# Food Research

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***Volume 24, Number 3*** ***May-June, 1959***

AN OFFICIAL PUBLICATION OF

THE INSTITUTE OF FOOD TECHNOLOGISTS

# Food Research

*An Official Publication of*

**The Institute of Food Technologists**

Volume 24 MAY–JUNE, 1959 Number 3

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*Published Monthly by the*

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FOOD TECHNOLOGY

THE GARRARD PRESS

510-522 North Hickory Street

CHAMPAIGN, ILLINOIS

Volume 24 MAY–JUNE, 1959 Number 3

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Single copies of current volume: Domestic, $1.50; foreign, $1.75.

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# THE IDENTITY OF YEASTS IN THE SURFACE FLORA OF PACKAGED FRANKFURTERS[a]

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(Manuscript received August 12, 1958)

In the study of the microbial flora of commercially packaged frankfurters *(5)* yeasts were frequently encountered although the isolation medium was not one which would selectively favor yeasts. 22% of all isolates were yeasts and these were isolated from 56% of all samples.

Cured meats have long been known to harbor yeasts of the genus *Debaryomyces.* Cesari *(2)* and Cesari and Guilliermond *(3)* in 1919 and 1920 described but did not name yeasts from slimy sausages. Seven of their strains were later identified by Lodder and Kreger-Van Rij *(7)*, 5 were *Debaryomyces hansenii* and two were *Debaryomyces kloeckeri.* In 1938 Mrak and Bonar *(8)* described yeasts from slimy wiener sausages, which they identified as *Debaryomyces guilliermondii* var. *nova zeelandicus.* This is classified as *D. hansenii* by Lodder and Kreger-Van Rij. Costilow, Etchells and Blumer *(4)*, in 1954, described 89 yeasts from commercial meat brines (70 from surface films and 19 from subsurface samples). Three were *D. kloeckeri* and 86 were *Debaryomyces membranaefaciens* var. *hollandicus.* The latter strains assimilate lactose and would be classified as *D. hansenii* by Lodder and Kreger-Van Rij. Ogilvy and Ayres *(9)* found yeasts to be common on frankfurters, but they did not identify any of their isolates. Wickerham, in 1957 *(12)*, reported the occurrence of nitrite-assimilating *Debaryomyces* in 9 out of 10 samples of lunch meats.

## EXPERIMENTAL METHODS

The isolation procedure has been described previously *(5)*. The isolates were maintained in MY broth *(6)*.

The methods described by Wickerham *(11)* were used for determining sporulation, morphology, carbon and nitrate assimilation, fermentation, salt tolerance, gelatin liquefaction, growth temperatures, and starch production. Lipolytic ability and litmus milk reactions were determined as described by Lodder and Kreger-Van Rij *(7)*.

Carbon and nitrate assimilation were also determined using an agar plate method described by Benham *(1)*. Yeast carbon base (Difco) or yeast nitrogen base (Difco) and special agar, Noble (Difco), plus the required amount of carbon source or nitrogen source were used. The sugars were autoclaved separately and added aseptically before pouring the plates. The carbon base, nitrogen base, and nitrate were Seitz filtered and added to the sterile agar before pouring the plates. The plates were streaked from 3-day cultures of the yeasts in the yeast carbon base or yeast nitrogen base. The basal agar medium was also streaked for comparative purposes.

All identifications were made according to Lodder and Kreger-Van Rij *(7)*.

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[a] Journal Paper No. 165, American Meat Institute Foundation. This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, QM Research and Engineering Command, U. S. Army, and has been assigned No. 937 in the series of papers approved for publication.

## RESULTS AND DISCUSSION

Of the 78 strains of yeasts which were isolated from 8 brands of frankfurters, 22 were picked randomly for identification. Seven strains which were isolated from frankfurters which had received radiation doses of 0.2-0.5 megarads were also identified. The identity of these 29 isolates is summarized in Table 1.

**TABLE 1**

**Identity of yeast strains isolated from the surface of packaged frankfurters**

| Identification | No. of strains |
|---|---|
| *Debaryomyces kloeckeri* | 7 |
| *Debaryomyces hansenii* | 3 |
| *Debaryomyces subglobosus* | 3 |
| *Debaryomyces nicotianae* | 2 |
| *Candida lipolytica* | 2 |
| *Candida zeylanoides* | 2 |
| *Candida catenulata* | 2 |
| *Candida* sp. | 3 |
| *Torulopsis candida* | 2 |
| *Torulopsis gropengiesseri* | 1 |
| *Trichosporon pullulans* | 2 |

Members of the genus *Debaryomyces* were most common. They represented 4 of the 5 species recognized by Lodder and Kreger-Van Rij. The main difference between these 4 species is in the presence or absence of rapid pellicle formation and in lactose assimilation. All except *D. subglobosus* have been described previously as occurring on cured meats. Only one *Debaryomyces*, a strain of *D. subglobosus*, was isolated from irradiated frankfurters.

*Torulopsis* species have not been described previously as meat spoilage organisms and they were not encountered frequently in this study. One of the 2 *T. candida* was from irradiated frankfurters.

*Candida* strains have been isolated from fresh meats by Scott *(10)* but there is no reference to their occurrence on cured meats. The 3 strains designated in Table 1 as *Candida* species did not appear to belong to any of the species described by Lodder and Kreger-Van Rij *(7)*. A pellicle was rapidly formed in broth cultures. The cells were fairly large and round to oval. A small to moderate amount of pseudomycelium of the *Candida* type was produced. Giant cells were present. No sporulation was observed. Nitrate was not assimilated. Glucose, sucrose, and maltose were fermented. Maltose was very weakly fermented. Lactose and raffinose were not fermented. Glucose, galactose, maltose, and sucrose were assimilated and lactose was not. Good growth occurred and a pellicle was formed with ethanol as the sole carbon source. Four of the 9 *Candida* were from irradiated samples, including both *C. catenulata*, 1 *C. zeylanoides*, and 1 *C. sp.*

The stock cultures of the two strains of *Trichosporon pullulans* dissociated. One showed two colony types and the other three. These were reisolated and their characteristics compared with the original types. The dissociates differed from the parent strains in that the colony was "rougher" and somewhat more mycelium was formed. One sub-strain had lost the

ability to liquefy gelatin and to peptonize litmus milk. In all other respects they were identical to the parent strains. One of these *T. pullulans* strains was isolated from irradiated frankfurters.

The determination of nitrate assimilation using Benham's plate method agreed 100 % with Wickerham's tube method and had the advantage of being much faster.

Use of this method to determine sugar assimilation was also successful. In all cases when there was growth on the agar plate there was growth in the liquid medium. However, in some cases where the growth in the broth was late or quite weak, there was no growth on the plate. The plate method therefore could be used routinely only if the negative results were checked by using the liquid medium.

Yeasts of the genus *Debaryomyces* are unable to utilize nitrate as their sole source of nitrogen. Wickerham *(12)* described yeasts of this genus which were isolated from lunch meats that could utilize nitrite as their nitrogen source. All of the strains of yeasts in this study which were nitrate negative were tested for this property. Both of the strains of *Torulopsis candida* utilized nitrite and none of the *Candida* strains did. Some, but not all, of the strains of *Debaryomyces* had this ability, as may be seen in Table 2.

**TABLE 2**

**Nitrite utilization by yeast strains from frankfurters [1]**

| Yeast species [2] | No. of strains tested | No. that use nitrite |
|---|---|---|
| *Debaryomyces kloekeri* | 7 | 4 |
| *Debaryomyces hansenii* | 3 | 2 |
| *Debaryomyces subglobosus* | 3 | 1 |
| *Debaryomyces nicotianae* | 2 | 1 |
| *Torulopsis candida* | 2 | 2 |
| *Torulopsis gropengiesseri* | 1 | 0 |

[1] None of these strains were able to utilize nitrate.
[2] The 9 strains of *Candida* listed in Table 1 were unable to utilize either nitrate or nitrite. The 2 strains of *Trichosporon* utilized both of these nitrogen sources.

Seitz filtered $KNO_2$ was added to yeast nitrogen base (Difco) to give a final concentration of 0.05% or 0.005%. All of the yeasts were transferred again in this medium. Agar plates of the same medium were also streaked. The same results were obtained in all cases. The nitrite negative yeasts did not utilize this as a nitrogen source even after 32 days of incubation in media with a pH from 4.8 to 6.5. Heavy growth rapidly occurred in the medium with 0.05% $KNO_2$ within 3 days with the yeasts which utilized nitrite. This concentration definitely inhibited the other yeasts.

All of these yeasts which were isolated from frankfurters are peculiarly adapted to growth on cured meats. All grew at temperatures down to at least 2° C. The strains of *Debaryomyces* and *Torulopsis* could grow in media containing 20% salt. The *Candida* culture tolerated 10-15% salt, and the *Trichosporon pullulans* strains tolerated 7.5% salt. Two species had marked lipolytic ability — *Trichosporon pullulans* and *Candida lipolytica*. The ability of certain strains to utilize nitrite also probably enhances their ability to grow on cured meats. Although few cultures were studied, it appears that irradiation eliminated *Debaryomyces* more readily than *Candida*.

**SUMMARY**

Twenty-nine strains of yeasts isolated from frankfurters were identified. These were members of the genera *Debaryomyces, Torulopsis, Candida,* and *Trichosporon.* Half of the strains of *Debaryomyces* were able to utilize nitrite but not nitrate as their sole source of nitrogen. The two strains of *Torulopsis candida* were also able to utilize nitrite but not nitrate.

LITERATURE CITED

1. BENHAM, R. W. The genus *Cryptococcus*: The present status and the criteria for the identification of the species. *Trans. N. Y. Acad. Sci.,* **17,** 418 (1955).
2. CESARI, E. P. La maturation des saucissons. *Compt. Rend. Acad. Sci.,* **168,** 802 (1919).
3. CESARI, E. P., AND GUILLIERMOND, A. Les levures des saucissons. *Ann. de l'Inst. Pasteur,* **34,** 229 (1920).
4. COSTILOW, R. N., ETCHELLS, J. L., AND BLUMER, T. N. Yeasts from commercial meat brines. *Appl. Microbiol.,* **2,** 300 (1954).
5. DRAKE, S. D., EVANS, J. B., AND NIVEN, C. F., JR. The microbial flora of packaged frankfurters and their radiation resistance. *Food Research,* **23,** 291 (1958).
6. HAYNES, W. C., WICKERHAM, L. J., AND HESSELTINE, C. W. Maintenance of cultures of industrially important microorganisms. *Appl. Microbiol.* **3,** 361 (1955).
7. LODDER, J. AND KREGER-VAN RIJ, N. J. W. *The Yeasts, A Taxonomic Study.* North Holland Publishing Co., Amsterdam, Holland (1952).
8. MRAK, E. M. AND BONAR, L. A note on yeast obtained from slimy sausage. *Food Research,* **3,** 615 (1938).
9. OGILVY, W. S. AND AYRES, J. C. Post-mortem changes in stored meats. V. Effect of carbon dioxide on microbial growth on stored frankfurters and characteristics of some microorganisms isolated from them. *Food Research,* **18,** 121 (1953).
10. SCOTT, W. J. The growth of microorganisms on ox muscle. *J. Council Sci. Ind. Research,* **9,** 177 (1936).
11. WICKERHAM, L. J. *Taxonomy of Yeasts. Tech. Bull.* 1029, U. S. Dept. of Agriculture, Washington, D. C. (1951).
12. WICKERHAM, L. J. Presence of nitrite assimilating species of *Debaryomyces* in lunch meats. *J. Bacteriol.,* **74,** 832 (1957).

# EFFECT OF ANTIBIOTICS ON THE THERMAL DEATH RATE OF SPORES OF FOOD SPOILAGE ORGANISMS

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(Manuscript received August 9, 1958)

The possibility of using antibiotics in the preservation of food has been under study by various workers for some years. Among the applications considered have been use in conjunction with mild heat for preservation in hermetic containers, as a surface treatment of perishable raw commodities, and to reduce the thermal resistance of spoilage bacteria. A review of the literature on the applications of antibiotics in food preservation has been presented elsewhere *(1)*. The referenced review contained a section on the influence of antibiotics on the thermal resistance of spoilage bacteria. Since the review was published in 1955, other work *(4)* has been reported to show that certain of the food spoilage bacteria are appreciably less heat resistant when heated in the presence of antibiotics.

The work reported in this paper, completed in 1953, was undertaken to evaluate several antibiotics that appeared to show promise. The thermal-death-time tube *(2)* approach to the antibiotic-heat resistance test has not been described in published papers so far as the authors are aware. The results of such an approach to the problem of heat-antibiotic synergism are reported herein.

## EXPERIMENTAL PROCEDURE

Antibiotics tested were subtilin (Merck & Company, Inc.), tyrothricin, methylol gramicidin (S. B. Penick & Company), and rhatany root (also from S. B. Penick & Company) prepared as an extract in the N.C.A. Laboratory. The concentration of the antibiotic in the substrate was usually 20 p.p.m. This concentration was prepared on a weight basis except in the case of subtilin where a 100% activity basis was used. Subtilin stock suspension was prepared in distilled water; tyrothricin and methylol gramicidin were dissolved first in ethanol and subsequently added to water. Rhatany root extract, prepared as outlined in U. S. Patent No. 2,550,256, was dissolved in ethanol. Ethanol controls showed that the quantity of ethanol used was not inhibitory to the spoilage organisms on test.

Spoilage organisms tested were putrefactive anaerobe No. 3679 and *Bacillus stearothermophilus* (flat sour) No. 1518 of the N.C.A. Laboratories, (Table 1). The putrefactive anaerobe spore suspension was prepared by the method of Reed, Bohrer, and Cameron *(5)*. The No. 1518 spore suspension was prepared by inoculating Kolle flasks of nutrient agar with a water suspension of one-day-old cultures of the microorganisms. After 72 hours growth at 131° F. (55° C.), the spore crop was harvested with sterile water and filtered through sterile cheesecloth.

The substrates in which the antibiotic and spore suspension were heated were pea brine and corn brine obtained from cans of the respective products processed on a single day of a commercial pack. The pea brine had a pH of 6.0 and the corn brine a pH of 6.37 as determined by a Beckman glass electrode pH meter. The pea brine had a density of 8.4° Brix and the corn brine 10.7° Brix. Salt content was 0.65% for the peas and 0.85% for the corn.

The heating method used for comparing spore destruction rates of the food spoilage organisms was that of Esty and Williams *(2)* using a constant temperature oil bath. Three temperature variables were used: 212°, 229.6°, and 240° F. Pyrex tubes of 7 mm. inside

**TABLE 1**

**Variables used in experimental tests**

| Spore suspension | Antibiotic [1] | Concentration (ppm) | Substrate | Temperature of heating (° F.) |
|---|---|---|---|---|
| F. S. No. 1518 | Subtilin | 20 | Pea brine | 212 |
| F. S. No. 1518 | Subtilin | 20 | Pea brine | 229.6 |
| F. S. No. 1518 | Subtilin | 20 | Pea brine | 240 |
| F. S. No. 1518 | Methylol Gramicidin | 20 | Pea brine | 240 |
| F. S. No. 1518 | Rhatany Root Extract | 20 | Pea brine | 240 |
| F. S. No. 1518 | Tyrothricin | 20 | Pea brine | 240 |
| F. S. No. 1518 | Tyrothricin | 5 | Pea brine | 240 |
| F. S. No. 1518 | Tyrothricin | 20 | Corn brine | 240 |
| F. S. No. 1518 | Subtilin | 20 | Corn brine | 240 |
| P. A. No. 3679 | Subtilin | 20 | Pea brine | 229.6 |
| P. A. No. 3679 | Methylol Gramicidin | 20 | Pea brine | 229.6 |
| P. A. No. 3679 | Rhatany Root Extract | 20 | Pea brine | 229.6 |
| P. A. No. 3679 | Tyrothricin | 20 | Pea brine | 229.6 |

[1] A control with no antibiotic material was included in each of the tests.

diameter were used in lengths of about 6 inches. The times for withdrawal of the tubes from the oil bath were set at 0, 5, 10, 15, 30, 60, 90, and 120 minute periods. Due allowance was made for heat penetration lag.

The procedure for the thermal death rate tests was as follows: Spores were added to either corn or pea brine to give about 10,000,000 spores per milliliter. This high concentration was used to permit spore recovery after the antibiotic was diluted below an inhibitory concentration. The spore-brine suspension was then divided into two equal portions. Concentrated antibiotic solution was added in amounts of less than 1 ml. to one of these portions. Each portion was pipetted into pyrex tubes in approximately 2 ml. amounts and the tubes were heat sealed. Three test tubes containing antibiotic and three control tubes were withdrawn at the completion of each heating time interval in the oil bath and immediately cooled. The contents of the respective tubes were composited, and dilutions of each test and control liquid were immediately made in sterile water.

Putrefactive anaerobe No. 3679 was subcultured in deep agar shake tubes described by Miller, Garrett, and Prickett *(3)*. The freshly exhausted medium [4] was of the following composition:

| | | |
|---|---|---|
| Tryptone | 10 | grams |
| Dextrose | 1 | gram |
| $K_2H\ PO_4$ | 1.25 | grams |
| Yeast extract | 1.0 | gram |
| Beef extract | 3.0 | grams |
| Soluble starch | 1.0 | gram |
| Sodium thioglycollate | 0.5 | gram |
| Agar | 15 | grams |
| Water | 1 | liter |

Subculture tubes were stratified with sterile vaseline and incubated at 85° F. for 3 weeks. However, since there was no significant increase in number of colonies developed after 5 days and gas sometimes developed later, the spore counts reported are essentially those obtained at the end of a 5-day incubation period.

The dilution counts on putrefactive anaerobe No. 3679 are averages of colony counts in tubes showing below 100 colonies per tube. The percent survivors were calculated from the respective zero counts of test and control dilutions.

*B. stearothermophilus* No. 1518 spore dilutions were subcultured by plating one milliliter of each dilution in triplicate. The plating medium was Difco dextrose tryptone agar. In-

[4] The pH of the medium was normally 7.2 and hence did not have to be adjusted.

cubation was at 131° F. for 3 days. Longer incubation in containers which prevented evaporation did not increase the counts.

The dilution counts for flat sour No. 1518 are averages of colony counts on plates showing 30 to 300 colonies. Percent survivors were calculated from the zero sample count of the antibiotic-free material for both the control and the antibiotic test. Had this procedure not been used, the tests curves would very nearly approach the control curves. This procedure was used because it was noted that the mere addition of antibiotic lowered the spore count of this organism considerably, even though the antibiotic was subsequently diluted beyond the inhibitory point.

In plotting results with both organisms, the log of percent survivors was plotted against the heating time in minutes. Variables in the experimental tests are listed in Table 1.

## RESULTS AND DISCUSSION

The survivor curves plotted for putrefactive anaerobe No. 3679 spores heated in pea brine at 229.6° F. (110° C.) with and without 20 p.p.m. of each of the antibiotics are shown in Figure 1. The shaded area represents

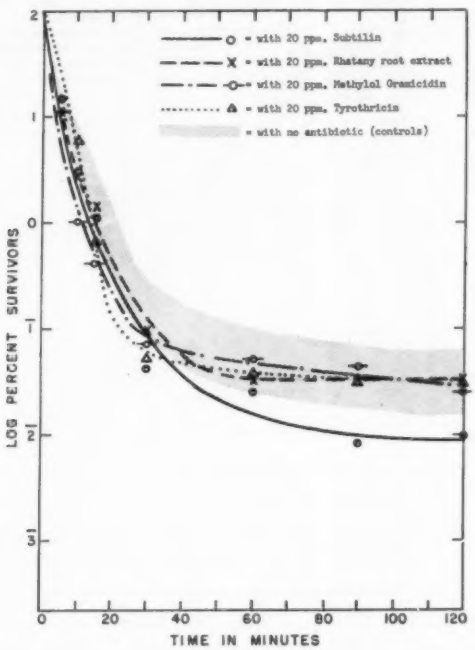


**Figure 1. Survivor curves of Putrefactive Anaerobe No. 3679 in pea brine at 229.6° F. with and without antibiotics.**

the upper and lower limits obtained in the four control tests. Subtilin was the most effective of the four antibiotics in increasing the destruction rate of putrefactive anaerobe No. 3679. However, at 0.1% survival a difference of only 5 minutes is noted between the subtilin and control. This slight advantage in destruction when interpreted in terms of required process

time at usual processing temperature of 240° F. would have no significant effect. The subtilin curve, even at the 120 minute point, parallels the control curves, and it is apparent that the distance between the limits of the four control tests is greater than the deviation of the subtilin curve from the lower limit of the control curve. One could consider the addition of the antibiotic advantageous in hastening spore destruction only if the subtilin curve had continued to recede downward from the control curves. It might be assumed that the action of subtilin in this case was diminished by the longer heating at the high temperature. It is apparent that the antibiotics in the concentrations tested had no striking effect in increasing the destruction of spores of putrefactive anaerobe No. 3679.

The destruction rates plotted for *B. stearothermophilus* spores heated at 240° F. (116° C.) in pea brine with and without 20 p.p.m. of each of the antibiotics are shown in Figure 2. It will be noted that the survivor curves

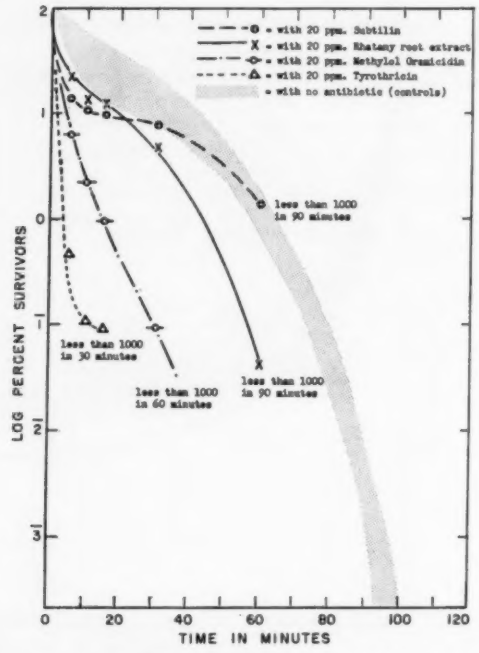


**Figure 2. Survivor curves of Bacillus stearothermophilus No. 1518 in pea brine at 240° F. with and without antibiotics.**

representing antibiotic-containing substrates show a more rapid initial destruction rate than do the 4 control tests (shaded area). However, the survivor curve for subtilin very nearly approaches that of the controls. The survivor curves indicate that three of the antibiotics tested accelerated the rate of destruction of flat sour spores, with tyrothricin showing the most pronounced

effect. It is to be noted that the final spore recovery points plotted for the antibiotic tests represent survival of 1,000 or more spores. The dilution required to eliminate residual inhibitory effects precluded defining the curves beyond these points.

Subtilin was tested at 212° F., and 229.6° F. as well as at 240° F. against "flat sour" organism No. 1518. No significant difference in the thermal death rate in test and control experiments was found at the two lower temperatures. The antibiotic seems effective during the initial heating period and seems to lose its "killing" power during the later heating period. Potency tests have indicated that subtilin tends to lose some of its effect upon severe heating.

The effect of reduced concentrations of tyrothricin is shown in Figure 3.

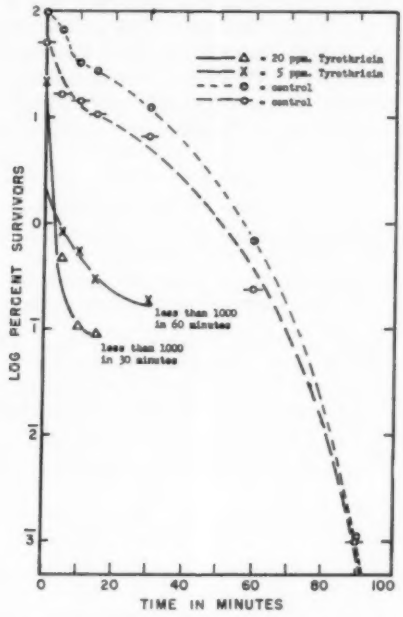


**Figure 3. Survivor curves of Bacillus stearothermophilus No. 1518 in pea brine at 240° F. with different concentrations of Tyrothricin.**

Even at 5 p.p.m. tyrothricin appears to accelerate the destruction rate of "flat sour" spores. A concentration of greater than 20 p.p.m. could not be tested by our method because the antibiotic concentration could not be diluted below its inhibitory level within the range of recoverable spore numbers.

The tests described for "flat sour" organism No. 1518 in pea brine were also conducted in corn brine. There was no significant difference in the results obtained in the two substrates.

It is apparent that the antibiotics tested are more effective in increasing

the destruction rate of the flat sour spoilage type *(B. stearothermophilus)* than the putrefactive spoilage type (P.A. No. 3679). Putrefactive anaerobic spoilage types include *Cl. botulinum,* which is the organism of health significance in canning, as opposed to the flat sour spoilage types which are only of economic significance. In view of this, it appears that the addition of an antibiotic, such as tyrothricin, might promote effective "complete" sterilization by a given heat process but would not decrease the minimum processing requirement for commercial sterility.

## SUMMARY

A series of experiments were conducted to compare the thermal death rate of spores of putrefactive anaerobe No. 3679 and *B. stearothermophilus* No. 1518 in the presence and absence of the antibiotics subtilin, tyrothricin, methylol gramicidin, and rhatany root extract. The results obtained indicated that the thermal death rate of spores of organism No. 1518 is accelerated by the addition of three of the four antibiotics tested. The antibiotics did not affect the thermal death rate of spores of putrefactive anaerobe No. 3679 to a significant degree.

## LITERATURE CITED

1. Campbell, L. L., Jr. and O'Brien, R. T. Antibiotics in food preservation. *Food Technol.,* **9,** 461 (1955).
2. Esty, J. R., and Williams, C. C. Heat resistance studies I. A new method for determination of heat resistance of bacterial spores. *J. Infect. Diseases,* **34,** 516 (1924).
3. Miller, N. J., Garrett, O. W. and Prickett, P. S. Anaerobic technique — a modified deep agar shake. *Food Research,* **4,** 447, (1939).
4. O'Brien, R. T., Titus, D. S., Devlin, K. A., Stumbo, C. R., and Lewis, J. C. Antibiotics in food preservation II. Studies on the influence of subtilin and nisin on the thermal resistance of food spoilage bacteria. *Food Technol.,* **10,** 352 (1956).
5. Reed, J. M., Bohrer, C. W., and Cameron, E. J. Spore destruction rate studies on organisms of significance in the processing of canned foods. *Food Research,* **16,** 383 (1951).

# APPLICATION OF FLAVOR ENZYMES TO PROCESSED FOODS II. COMPARISON OF THE EFFECT OF FLAVOR ENZYMES FROM MUSTARD AND CABBAGE UPON DEHYDRATED CABBAGE [a]

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(Manuscript received May 31, 1958)

A research program on the application of flavor enzymes to cabbage, supported in these laboratories both by the Quartermaster Pioneering Research Division, Natick, Massachusetts, and by the Quartermaster Food and Container Institute for the Armed Forces, Chicago, Illinois, showed that objective methods for the assay of enzymes, particularly those from different sources, were needed. Subsequent investigations under the sponsorship of Evans Research, reported herein, indicated that paper and gas chromatography offer much hope for the solution of this and similar problems. This study is part of a larger program at Evans Research and Development Corporation on flavor-producing enzymes. The basic concept and earlier results have been reported previously *(4, 5, 8)*.

Thioglucosidases [b] are present in enzymes obtained from cabbage and mustard, and they can be conveniently assayed for by their glucose-releasing action on sinalbin [c] and sinigrin [d]. Sensory panel studies, however, indicated that enzyme preparations from cabbage and mustard could be distinguished by their effect on flavor formation in reconstituted dehydrated cabbage. In view of the importance of isothiocyanates (mustard oils) to cabbage flavor, it was felt that a better picture of enzyme activity would be obtained by assaying for the released mustard oils, either directly by gas chromatography, or indirectly, by paper chromatographic examination of the thioureas derived from the mustard oils, according to the method of Kjaer and Rubinstein *(6)*.

## PAPER CHROMATOGRAPHIC EVALUATION OF EFFECT OF ENZYMES ON DEHYDRATED CABBAGE

**Materials and Methods.** Accordingly, the effects of an enzyme preparation from mustard seed and an enzyme preparation from cabbage leaves were compared by paper chromatographic techniques. Three samples were used for this study:

1. dehydrated cabbage plus cabbage enzyme
2. dehydrated cabbage with no enzyme
3. dehydrated cabbage plus mustard enzyme

The mustard enzyme was obtained from 1500 g. of Montana Brown mustard seed (1956 crop). The seeds were crushed, defatted with petroleum ether, and extracted with water. The enzyme fraction was obtained by precipitation with an equal volume of very cold acetone.

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[a] Presented at the Eighteenth Annual Meeting of IFT, Chicago, Illinois, May 27, 1958.

[b] The name, "myrosinase" or "sinigrinase", is sometimes used for the thioglycosidase in black mustard. This enzyme hydrolyzes the thioglucoside, potassium myronate or sinigrin, found in black mustard.

[c] Chief thioglucoside found in white mustard.

[d] Chief thioglucoside found in black mustard.

The cabbage enzyme was prepared from the leaves of the Danish Ball-Head Cabbage. Separate enzyme preparations were made from the inner and outer leaves, the core and the seed, but sensory panel studies indicated the choice of enzymes from the leaves for this work.

The dehydrated cabbage was commercially dehydrated Copenhagen cabbage, obtained through the courtesy of Dr. Fred Heiligman of the Quartermaster Food and Container Institute for the Armed Forces, Chicago.

The enzyme fractions used were assayed for nitrogen content and for activities in hydrolyzing sinalbin and sinigrin. The amounts of enzyme preparation added were calculated to contain the same weight of nitrogen.

Each of the 3 samples was prepared by the following procedure: Using a 12-liter round bottom flask and a mechanical stirrer, 500 g. of dehydrated cabbage (equivalent to about 22 lb. of fresh cabbage) was blended with 10 liters of distilled water. Then, for Samples 1 and 3, the respective enzyme was added (4 grams in 50 ml. of distilled water) to the cabbage slurry. An infrared lamp was used to maintain the slurry at a temperature of 30° C. during the reaction time. After 6 hours, the stirring was stopped, and the slurry was allowed to stand for 16 hours at room temperature under a layer of toluene.

The slurry was then extracted with 6 liters of n-hexane, in 3 equal portions. Each two-liter portion of n-hexane was added to the slurry and stirred mechanically for 2 hours. The n-hexane layer was siphoned off, and another 2-liter portion of fresh n-hexane was added to the slurry. Infrared analysis of a sample from the first 2-liter portion of n-hexane extract detected the presence of isothiocyanates. Isothiocyanates were not detected in the next n-hexane extract. Therefore, 3 extractions were considered adequate for removal of isothiocyanates from the slurry.

The n-hexane extract was concentrated from 6 liters to about 500 ml. by distillation on a hot water bath. Examination of samples of this n-hexane extract by means of infrared analysis and gas chromatography indicated the presence of mustard oils, particularly allyl isothiocyanate.

To the 500 ml. of concentrated n-hexane extract of the cabbage slurry was added 100 ml. of concentrated ammonium hydroxide and 100 ml. of ethanol. The solution was agitated and then allowed to stand at room temperature for 20 hours. Then it was concentrated to 2 ml. (by distillation on a hot water bath) for examination by paper chromatography.

Paper chromatography of the various fractions was carried out using Whatman No. 1 paper which had been equilibrated for 16 hours in an atmosphere of chloroform and water. The mobile phase was chloroform saturated with water. Ultraviolet light, platinic iodide *(2)*, and sodium azide-iodine reagent *(10)* were the visualizing agents used to detect the isothiocyanates after their conversion to thioureas by treatment with concentrated ammonium hydroxide.

The sodium azide-iodine reagent was the most sensitive reagent, but it was not very specific. Platinic iodide, used as a dipping bath rather than a spray, was also very sensitive, and rather more specific. It was found to be most reliable for this work if the concentration was three times higher than that recommended, and if the acetone was not added until just prior to use. Grote's reagent, which has been recommended for the detection of thioureas, was tried, but found unsatisfactory. A recent publication *(3)* also notes that Grote's reagent was less satisfactory than the sodium azide-iodine reagent for detection of similar sulfur-containing compounds on paper chromatograms.

A schematic diagram representing the results of several chromatograms is given in Figure 1.

All 3 samples of dehydrated cabbage gave a very weak positive reaction at the origin. Six additional corresponding reaction zones were detected for Samples 1 and 3, the enzyme-treated ones, indicating that no mustard oils were released in the untreated cabbage. These zones had the following $R_f$ values, relative to that of N-sec-butyl thiourea, and intensities: 0.02, weak; 0.11, very weak; 0.42–0.50, very strong (corresponds to an authentic sample of N-allyl thiourea); 0.85, medium; 0.98, weak; and 1.11, very weak. The reaction zones at 0.42-0.50, 0.85, and 0.98 were the only areas which showed appreciable absorption under ultraviolet light, which is characteristic of thioureas. The concentrations of the other reaction zones may have been too low for such detection. In any case, there appeared to be no qualitative difference between Samples 1 and 3. Quantitatively, it could be seen that there were four components in Sample 3 that were present in smaller amounts in Sample 1. For

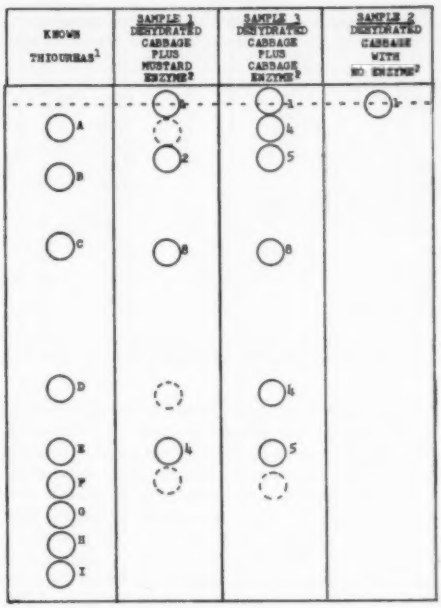


**Figure 1. Comparison of effect of cabbage and mustard enzymes on dehydrated cabbage using Whatman No. 1 paper, chloroform-water solvent system, and platinic iodide visualizing agent.**

**[1] See Table 1.**

**[2] The numbers refer to relative intensity: 1 = very very weak and 9 = very strong.**

example, when smaller and equal amounts of samples were examined, only three components could be found in Sample 1, but five were found in Sample 3. The compounds that were seen only when large amounts of the thiourea mixture were chromatographed are designated by dashed circles in Figure 1. In both samples, the major component was due to allyl thiourea. The identity of the others is uncertain, although certain possibilities (for example, 3-butenyl thiourea, sec-butyl thiourea, and $\omega$-methyl sulfide-n-propyl thiourea) are evident from a perusal of the $R_f$ values of authentic thioureas given in Table 1. To allow comparison with Kjaer's results *(6)*, all the $R_f$ values in this table have been given as absolute values.

To determine whether any mustard oils or similar compounds remained in the aqueous phase (because they had not been extracted by the hexane), the liquid was expressed from the n-hexane-extracted cabbage (which had been standing at room temperature for 16 hours) by filtering it through cheesecloth and squeezing to obtain as much as possible. A preliminary experiment had indicated that almost 95% of the water initially added to dehydrated cabbage could be recovered. The resulting liquid was filtered under suction through a Buchner funnel, using Celite filter aid. This liquid was not clear, but further filtration did not improve it. Therefore, this liquid was concentrated by distillation from about 10 liters to one liter.

The distillate from Sample 1 had no obvious odor. The distillate from Sample 2 had a strong sulfurous, cooked cabbage odor. The distillate from Sample 3 had a cabbage-like odor with sharp sulfurous overtones. The one liter of liquid expressed from the n-hexane-extracted cabbage slurry was divided in two. One half received no additional chemical

**TABLE 1**

**Average R values of known n-substituted thioureas [1]**

| Code | Thiourea | $R_f$ | Literature values (6) |
|---|---|---|---|
| A | Methyl | 0.02 | 0.03 |
| B | Ethyl | 0.11 | 0.12 |
| C | Allyl | 0.21 | 0.22 |
| D | 3-Butenyl | 0.48 | 0.48 |
| E | sec-Butyl | 0.54 | 0.61 |
| F | ω-Methyl sufide-n-propyl | 0.58 | — |
| G | n-Butyl | 0.60 | 0.70 |
| H | Benzyl | 0.63 | 0.75 |
| I | 4-Pentenyl | 0.71 | — |
| | Phenyl | 0.71 | 0.83 |
| J | β-Phenyl ethyl | 0.75 | 0.92 |
| | n-Hexyl | 0.84 | — |

[1] Whatman No. 1 paper, descending technique, and chloroform saturated with water irrigant.

treatment. The other 500 ml. was treated with 100 ml. of concentrated ammonium hydroxide and 100 ml. of ethyl alcohol, agitated, and allowed to stand for 20 hours at room temperature. Both samples were concentrated to a small volume for paper chromatography. This concentration step was limited by the tendency of the samples to become tars.

Paper chromatography detected one very weak reaction zone in both of the expressed liquid fractions. Apparently, treatment with ammonium hydroxide had no differentiating effect. The expressed liquids for Samples 1, 2, and 3 all had this same reaction zone. Although the reaction zone had the same $R_f$ value as that of an authentic sample of N-allyl thiourea, it was not detected by ultraviolet light. This might be a concentration effect.

In Figure 2, the mustard oils (as thioureas) obtained from cabbage enzyme-treated dehydrated cabbage are compared with those obtained in a previous study from fresh cabbage. The results are not strictly comparable, since the fresh cabbage (1,000 pounds) was worked up by a large-scale procedure, which is now known to cause the loss of allylisothiocyanate. This mustard oil is so volatile that it is lost completely when trying to recover it from large volumes of solvent by evaporation. For this reason, the dashed circle is included in Figure 2 to represent the allyl thiourea that would have been found if reaction with ammonia had been allowed to occur before working up of the solvent extract from fresh cabbage.

The differences noted are undoubtedly caused by the disparity of the methods and the amounts of cabbage used and possibly by differences in geographical origin. Also, the nature of the chromatographic system is such that reproducible absolute $R_f$ values are not easily obtained and it is difficult to compare substances that are not run on the same chromatogram. However, it is clear that a fairly close correlation can be made between the patterns given by fresh cabbage and those given by enzyme-treated dehydrated cabbage. Substantially, this same conclusion was reached by Bailey *et al.* *(1)* of the Quartermaster Research and Engineering Command, Natick, Massachusetts on the basis of mass spectroscopic data of the volatiles obtained from fresh, dehydrated, and mustard enzyme-treated dehydrated cabbage.

## ACTION OF CABBAGE AND MUSTARD ENZYME ON SINIGRIN AND SINALBIN

The same sample of mustard enzyme used in the paper chromatographic study was compared for thioglucosidase activity on two substrates with a corresponding sample of enzyme prepared from cabbage. Since the nitrogen contents of these crude preparations were 10.9% and 8.7%, respectively, the amounts used were adjusted to make possible a comparison of the assays on an equal nitrogen basis. The two substrates chosen were sinigrin and sinalbin. These thioglucosides were obtained by hot alcohol extraction of black and white mustard flour, respectively.

Samples were prepared so that complete hydrolysis of either substrate would yield equimolar amounts of glucose, that is, the substrate-enzyme ratio would be similar in all

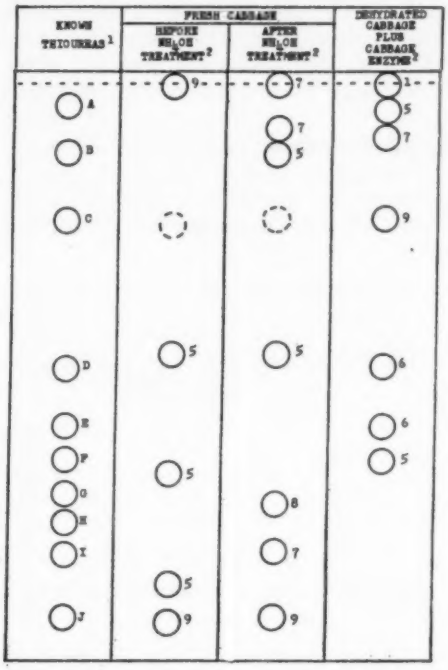


**Figure 2. Comparison of fresh cabbage with dehydrated cabbage after enzyme treatment using Whatman No. 1 paper, chloroform-water solvent system, and platinic iodide visualizing agent.**

**[1] See Table 1.**

**[2] The numbers refer to relative intensities: 1 = very very weak and 9 = very strong.**

cases. Each sample contained 0.27 mg. of nitrogen and was buffered to pH 5 with acetate buffer. Under these experimental conditions, a unit of enzyme activity was defined as that which released 0.1 mg. of glucose per hour from its substrate. The measurement of released glucose was made colorimetrically using the 3,5-dinitrosalicylic acid method of Sumner and Sisler *(11)*. The results obtained are shown in Table 2 and plotted in Figure 3.

**TABLE 2**

**Comparison of enzymatic activity on sinalbin and sinigrin**

| Enzyme preparation from | Activity on Sinalbin (units/mg N) | Activity on Sinigrin (units/mg N) | Ratio [1] |
|---|---|---|---|
| Cabbage | 27 | 20.4 | 1.34 |
| Mustard | 11 | 18.5 | 0.6 |

[1] Ratio of activity on sinalbin to activity on sinigrin.

When sinalbin was used as the substrate, the cabbage enzyme showed 27 units activity per milligram of nitrogen; the mustard enzyme, only 11 units. When sinigrin was used as the substrate, the cabbage enzyme showed 20.4 units activity; the mustard enzyme, 18.5 units.

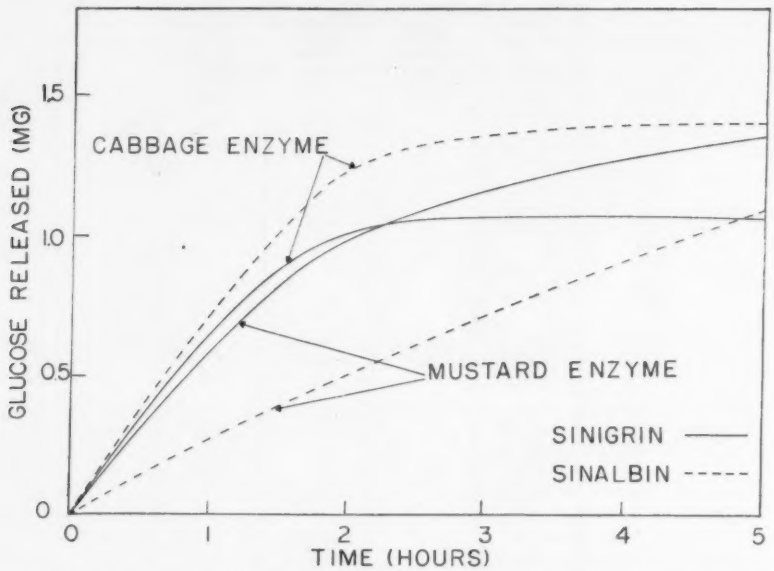


**Figure 3. Comparative activity of thioglucosidases from cabbage and mustard on sinigrin and sinalbin.**

Column 3 of Table 2 gives the comparative rates during the initial 2 hours of the reaction. These reveal that cabbage "myrosinase" has less effect on sinigrin than sinalbin, whereas exactly the opposite is true for the mustard "myrosinase".

A comparison of the reaction rates of thioglucosidases from white mustard and from *Aspergillus sydowi* has also been carried out recently *(9)*. This study showed that these thioglucosidases can be distinguished by this method.

The difference in relative activities on two substrates by two enzyme preparations might indicate that at least two thioglucosidases exist, one more active on sinigrin and the other on sinalbin. Another possible explanation is that there may be two or more enzymes with differences in affinity for the two substrates. These thioglucosidases may be present in the enzyme preparations in varying proportions.

Mustard, cabbage, and *Aspergillus sydowi* may contain different ratios of these thioglucosidases since preparations from all three have activity on both substrates, but their relative activities differ. The presence of glucose-using enzymes, such as glucose oxidase, in only one enzyme preparation would reduce the amount of glucose produced from both substrates and would not necessarily interfere with the ratio of activity.

Therefore, the important observation to be made from Table 2 is that the relative activities of mustard and cabbage enzyme preparations on two different thioglucosides differ sharply, pointing to a possible mechanism by which variations in mustard oil flavors may be produced in members of cruciferous plants.

## GAS CHROMATOGRAPHY OF MUSTARD OILS

An attractive alternative method for the analysis of isothiocyanates was offered by gas chromatography. Accordingly, the mixture of authentic mustard oils, as shown in Figure 4, was subjected to gas chromatographic

analysis under a variety of experimental conditions. Since these mustard oils vary greatly in volatility, certain compromises were necessary to obtain analyses within reasonable amounts of time. Thus, the final conditions selected are not necessarily those best suited for separation of any pair or group of isothiocyanates.

Very short (one-foot) columns were used to keep the length of exposure of the isothiocyanates to heat as short as possible, and to allow elution of the less volatile mustard oils at moderate temperatures. The column substrate was 20% di-n-octyl phthalate on 30-60 mesh crushed firebrick. The tem-

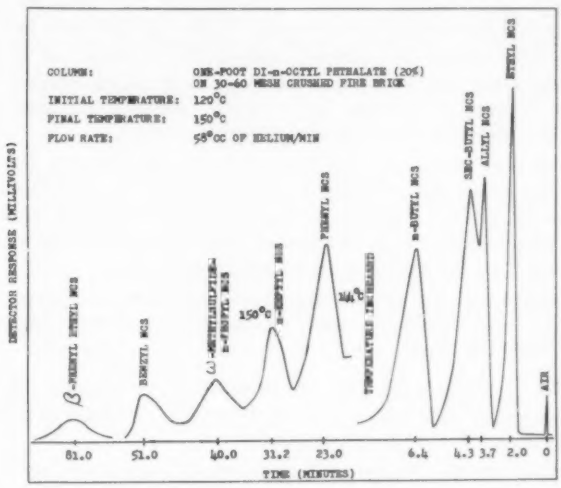


**Figure 4. Gas chromatogram of isothiocyanates.**

perature was increased during the run from 120° C. to 150° C., on account of the above factors. A recent publication *(7)*, which appeared since the start of this work, describes the separation of mustard oils at lower temperatures on a silicone grease column. However, only the more volatile mustard oils were examined.

The possibility of the inter-conversion of thiocyanates and isothiocyanates during gas chromatography was considered by using the same chromatographic conditions for the analysis of known thiocyanates. It was found that the retention times of thiocyanates were very close to those of the corresponding isothiocyanates, and that mixtures of thiocyanates and isothiocyanates were not resolved. However, infrared examination of isothiocyanates eluted from the columns, revealed that, under these chromatographic conditions, there is no sign of rearrangement to the thiocyanates. Likewise, the thiocyanates examined were not rearranged to the *iso* form. Allyl thiocyanate, which is likely to rearrange, was not examined, however, since the "authentic" sample obtained had already gone over to the isothiocyanate form.

It can be concluded, therefore, that gas chromatography offers an excellent route to direct examination and separation of naturally occurring mustard oils. In the particular cases under study, the amount of starting material was such that only allyl isothiocyanate was detectable as a result of enzyme action. Therefore, this approach was temporarily abandoned in favor of the more sensitive paper chromatographic route. It is seen, too, that identification of mustard oils by retention times alone may be hazardous, and infrared spectroscopy is a necessary confirmatory technique.

## SUMMARY

Cabbage and mustard enzyme preparations (in amounts calculated to contain the same weight of nitrogen) were allowed to react with equal portions of the same commercially dehydrated cabbage for 22 hours. The reaction mixture was extracted with n-hexane. This extract was treated with concentrated ammonium hydroxide to convert liberated mustard oils to thioureas for paper chromatographic examination.

At low concentrations, five components were detected for the cabbage enzyme, but only three of these were detected for the mustard enzyme. At higher concentrations, six similar components were detected for both enzymes. Four of these components were more strongly detected for the cabbage enzyme preparation than for the mustard enzyme preparation.

The comparison of the cabbage and mustard enzyme preparations, based on a paper chromatographic study of thioureas formed from released mustard oils, indicates that:

1. The same mustard oils are released from commercially dehydrated cabbage by cabbage and mustard enzyme. Mustard oils are not found in untreated dehydrated cabbage.
2. Apparently the mustard oils are released from commercially dehydrated cabbage at a greater rate, or to a greater extent, by the cabbage enzyme than by the mustard enzyme.

These findings may be correlated with intrinsic differences or relative affinities of the thioglucosidases in cabbage and mustard as indicated by comparison of enzymatic activities on sinigrin and sinalbin.

### Acknowledgments

The authors wish to thank Mr. John Hilovsky, Mr. John A. Seiler, Jr., Mr. Frank Ellison, and Miss Elinor Cohen for technical assistance.

## LITERATURE CITED

1. Bailey, S. D., Driscoll, J. L., and McCarthy, A. J. The volatile components of fresh cabbage and enzyme-treated dehydrated cabbage. Abstracts of papers presented at the Institute of Food Technologists Meeting, Pittsburgh, May, 1957.
2. Feigl, F., *Spot Tests in Organic Analysis*, 5th ed. 1956. Elsevier, New York, N. Y., p. 88.
3. Hanson, K. R., and Whitaker, D. R. Detection of phenylthiohydantoins on paper chromatograms. *Chem. & Ind.* (London) 1958, 43.
4. Hewitt, E. J., Mackay, D. A. M., Konigsbacher, K., and Hasselstrom, T. The role of enzymes in food flavors. *Food Technol.*, **10**, 487-489 (1956).

5. HEWITT, E. J., MACKAY, D. A. M., KONIGSBACHER, K. S., AND HASSELSTROM, T. Flavor propagation through enzymatic action. *Chemistry of Natural Food Flavors.* A symposium sponsored by the National Academy of Sciences — National Research Council, for the Quartermaster Food and Container Institute for the Armed Forces, and Pioneering Research Division, Quartermaster Research and Engineering Center, May 1957, pp. 86-100.
6. KJAER, A., AND RUBINSTEIN, K. Paper chromatography of thioureas. *Acta Chem. Scand.*, **7**, 528-536 (1953).
7. KJAER, A., AND JART, A. *iso*Thiocyanates. XXIX. Separation of volatile *iso*thiocyanates by gas chromatography. *Acta Chem. Scand.*, **11**, 1423 (1957).
8. KONIGSBACHER, K. S., HEWITT, E. J., AND EVANS, R. L. Application of flavor enzymes to processed foods. I. Panel studies. *Food Technol.*, **13**, 128-132 (1959).
9. REESE, E. T., CLAPP, R. C., AND MANDELS, M. A thioglucosidase in fungi. *Arch. Biochem. & Biophys.*, **75**, 228-242 (1958).
10. Adapted from the reagent of SCHMALL, M., WOLLISH, E. G., AND SHAFER, E. G. E. Chromatography of organic bases on multibuffered paper. *Anal. Chem.*, **28**, 1374 (1956).
11. SUMNER, J. B., AND SISLER, E. B. A simple method for blood-sugar (determination). *Arch. Biochem.*, **4**, 333-336 (1944).

# THE RELATIONSHIP BETWEEN THE QUALITY OF DEHYDRATED RAW BEEF AND THE ADENOSINE TRIPHOSPHATASE ACTIVITY AFTER STORAGE AT VARIOUS MOISTURE CONTENTS

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(Manuscript received August 28, 1958)

It has been shown in a previous paper *(4)* that dehydration by either the freeze-drying or the accelerated freeze-drying (AFD) process *(1, 3, 7)* did not substantially change the actomyosin of beef muscle and the associated adenosine triphosphatase (ATPase) activity was reduced but little. The present work was designed to discover whether during subsequent storage there is a loss of ATPase activity and if there is, whether or not it bears a definite relationship to deterioration in dehydrated meat as assessed by a taste panel.

If a partial loss of ATPase activity were related to the actomyosin, the results could indicate either slight damage to the whole or extensive damage to part of the actomyosin. Deterioration of texture occurs during storage and it seemed probable that this might be associated with changes in the protein. For example, it has been found that casein becomes insoluble when stored in the 'dry' state in presence of glucose *(5, 6)*. A convenient way of assessing quantitatively changes in beef actomyosin during storage would be by measurement of the residual ATPase activity.

The possible survival of enzymes during storage is of interest since they might contribute to the more rapid deterioration observed in dehydrated raw beef as opposed to that which has been precooked. ATPase may not be concerned in this effect, but its survival will indicate the presence of many other enzymes.

In general the storage life of dried foods increases as their moisture content decreases. Some data for dehydrated raw beef is available *(2, 8)* but the method of drying may have a considerable effect on the storage properties. Tests were therefore carried out in which raw beef, dried by the AFD process, was stored at various moisture contents. At intervals samples were examined organoleptically and their actomyosin-ATPase activity was determined.

## EXPERIMENTAL

### Materials and Methods

**Storage test.** Beef steaks used for this test were taken from a batch dried in a commercial-scale plant by the AFD method *(7)*. The steaks were cut into pieces about 2.5 × 2.5 × 0.9 cm. and mixed well to achieve uniformity of starting material. The pieces were adjusted to the various moisture contents (3%, 7% and 11% approximately) by exposure to an atmosphere of 95% R.H. and 37° C. until the appropriate weight increase had been obtained. The maximum time required was approximately 6 hours. The samples at each moisture level were nitrogen packed into a 4-gallon can and held for 3 days at 18° C. to equilibrate the moisture levels. After this period, the fat and moisture contents

were determined on samples from each can and the beef was nitrogen-packed as 50 g. units into A1 cans. A bag of filter paper containing 5 g. granular calcium oxide was placed in some as an in-can desiccant. After packing, the seams of the cans were rendered air-tight by treatment with a bituminous paint. Cans were then stored at −20° C, 18° C or 37° C. Samples removed from store were examined for ATPase activity and their organoleptic qualities. Occasionally the fat and moisture contents and the reconstitution properties were determined.

**Method of reconstitution.** Weighed pieces of beef were put into cold water and kept submerged by a clock glass. Water absorption was determined by weighing the pieces after the surface water had been removed by blotting. After 30 minutes immersion the reconstituted pieces were minced to give a homogenous sample and the fat and moisture contents determined. From these figures, the reconstitution curve could be plotted as g.water/g. non-fatty solids (NFS) against time. It is appreciated that the method is subject to slight errors owing to the leaching of soluble components.

**Taste panel.** The fully reconstituted samples were cooked by simmering in water until tender with the addition of salt only. The beef was served to a panel of trained tasters, isolated in individual cubicles illuminated with a red light so that colour changes could not be detected and affect the marks. The scoring system is shown in Table 1.

**TABLE 1**

**Taste panel score sheet**

| Score | Toughness | Juiciness | Intensity of beef flavour | Taints |
|---|---|---|---|---|
| 0 | Very tough | Very dry | Very weak or entirely masked | Very strong |
| 2 | Tough | Dry | Weak | Appreciable |
| 4 | Slightly tough | Slightly dry | Medium | Slight |
| 6 | Tender | Juicy | Full | None |

Palatability was marked on a scale of 1 to 9, in which 9 was equated with 'like extremely' and 1 with 'dislike extremely'; a mark of 5 implied 'neither like nor dislike'. On this scale samples of fresh meat prepared according to taste panel procedure were generally given scores of 7 to 8.

In addition to the other attributes a marking was given for palatability. The latter indicates only the opinion of the panel and cannot be accepted to be representative of the average consumer. The tasters tended to be critical of the quality of the samples and this, together with the simple method of cooking, suggests that the palatability of the product would be improved if served as part of a dish with appropriate seasoning. The marking is used as a guide to changes in the palatability of the beef with storage.

Before the storage-life of any foodstuff can be assessed, some means of deciding when it has become unpalatable is required. The method must be arbitrary, since the palatability of food is a subjective quality which will vary with method of cooking, appetite and discrimination of the consumer, etc. The time required for the score of any one attribute to fall to a given level could be taken as the storage-life. However, this method ignores changes in other attributes and also the development of taints of an unpleasant nature. In this work, a marking of 2.5 on the palatability scale has been chosen as defining the end of storage life (provided that subsequent scores were not higher), the palatability marking being accepted as an 'omnibus' score for all attributes. This figure may appear rather low but, as has been explained, the palatability rating would almost certainly be improved if the beef were cooked in an attractive fashion and assessed by a representative group of the population. Although this method of assessing storage life is open to criticism, it has the advantage of simplicity.

**Determination of ATPase activity.** The method of enzyme preparation was as described in a previous paper *(4)*. 0.3 g. of finely-minced lean beef taken from a well-mixed sample was macerated, washed and made up to 100 ml. with water. Determinations were carried out on 0.05, 0.10 and 0.15 ml. portions of the suspension. Blank determinations were also done in which enzyme or ATP or both were replaced by water. By this means an estimate was obtained of the amount of inorganic phosphorus produced by ATP, by the enzyme preparation, and by the reagents alone. Standards containing 3.1 and 6.2 $\mu$g. inorganic phosphorus per tube were assayed together with the other phosphate determinations. From these, and from the fat and moisture contents of the minced dried beef, the

average activity of each sample was found in terms of μg. phosphorus produced/min./mg. NFS. It should be noted that solids referred to here are those of the original beef, not those in the enzyme suspension.

## RESULTS

The results, both for the taste panel and ATPase activity (measured in μgP produced/min./mg. NFS), are shown in the 6 charts constituting Figure 1. There is a general tendency for deterioration in eating quality to follow the decrease in ATPase activity. There was no appreciable change in toughness or juiciness in any of the samples.

*Sample A (3% M.C., 37° C.).* Loss in palatability followed the trend of the ATPase graph very closely. After 6 months this sample had fallen almost to the critical palatability score, but in the next 6 months very little significant change occurred.

*Sample B (in-can desiccant, 37° C.).* A similar tendency was shown in this sample, though there was in general less deterioration than in the samples with high moisture content or sample A. The overall storage life was estimated at more than one year.

*Sample C (3% M.C. −20° C.).* Deterioration of the organoleptic attributes of this sample was very slight during the storage period and the storage life was markedly in excess of one year.

*Sample D (6.3% M.C., 37° C.).* Deterioration was comparatively rapid in this sample and the quality was reduced to the critical palatability score in three months. The latter followed the decrease in ATPase activity very closely.

*Sample E (10.8% M.C., 37° C.).* The graphs for this sample showed a marked similarity to those for sample D though sample E had a shelf life of only one month.

*Sample F (3.3% M.C., 18° C.).* This sample had a longer shelf life than those with a similar moisture content stored at 37° C. It is interesting to note that the graphs for this sample bear a marked similarity to those of sample C.

**ATPase activity.** In all samples the rate of loss of enzymic activity is shown to be dependent on moisture and temperature. Those with moisture contents of 3% or less lost little if any activity in the first 3 months. Thereafter the ATPase activity declined, the rate of loss being retarded by storage at −20° C. and accelerated at 37° C. The samples at 18° C. kept better than those at 37° C. with an in-can desiccant, which in turn deteriorated more slowly than a sample at 37° C. without a desiccant. The enzymic activity decreased rapidly in the wetter samples.

**Reconstitution.** The graphs in Fig. 2 are plots of water uptake for samples stored for ½, 3 and 6 months. For the sake of clarity the initial points have been omitted. Again, the samples with high moisture levels which were stored at a high temperature deteriorated most quickly in rate and extent of reconstitution, although occasional inversions in the order were found.

**Moisture determinations.** These were done periodically during the storage test and the results for sample B and the averages for the other samples are given in Table 2.

**TABLE 2**

**Moisture contents during storage**

| *Samples* | A | C | D | E | F |
|---|---|---|---|---|---|
| a. *Samples without in-can desiccant* | | | | | |
| Storage temperature | 37°C | − 20°C | 37°C | 37°C | 18°C |
| Average moisture content ($g.H_2O$/100 g. NFS) | 3.1 | 2.9 | 6.3 | 10.8 | 3.3 |
| Standard deviation | ± 0.62 | ± 0.61 | ± 1.21 | ± 1.01 | ±0.48 |
| b. *Samples with in-can desiccant (B)* | | | | | |
| Time (months) | ½ | 1 | 2 | 3 | 6 | 7 |
| Moisture content | 2.8 | 2.2 | 1.9 | 0.85 | 1.0 | 1.3 |

In addition to the above assessments, individual fibres were tested for contractility (4) and small pieces of beef for catalase activity *(4)* after storage for 16 months. The samples stored at −20° C. or 18° C. were still quite strongly contractile but none of the others showed activity. Catalase activity on the other hand was present in all samples except the wettest. A boiled sample as a control was shown to have no catalase activity.

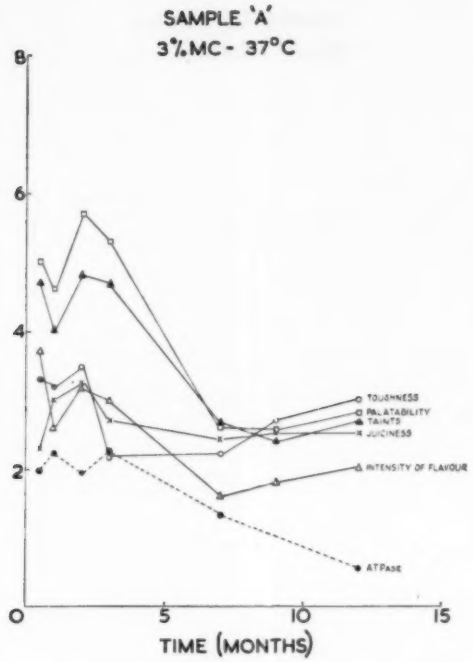


**Figure 1. Changes in dehydrated raw beef during storage. Sample 'A', ATPase activity is measured in $\mu$gP produced/min./mg.NFS. (Other graphs show taste panel scores hedonic scale).**

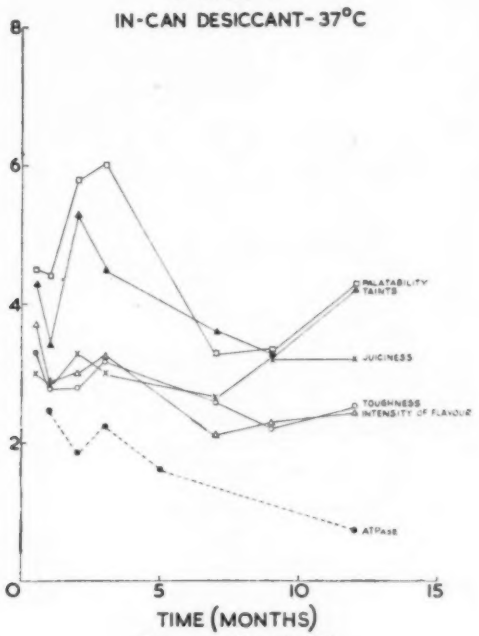


**Figure 1. Sample 'B'.**

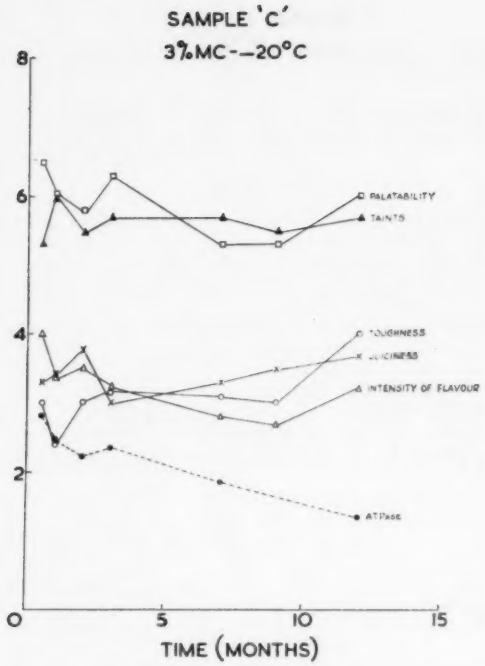


Figure 1. Sample 'C'.

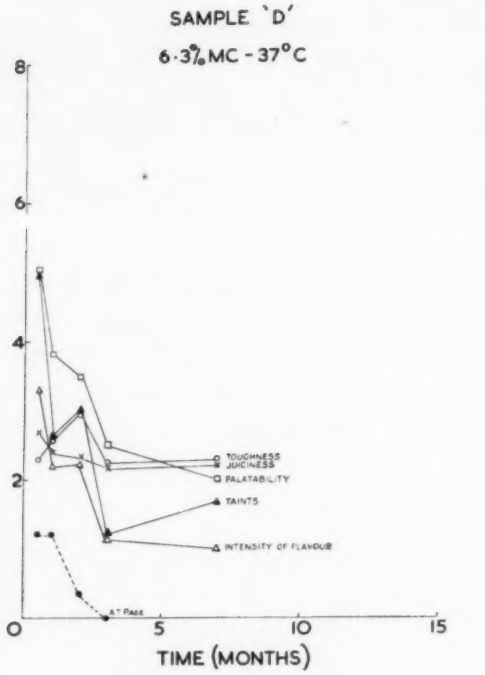


Figure 1. Sample 'D'.

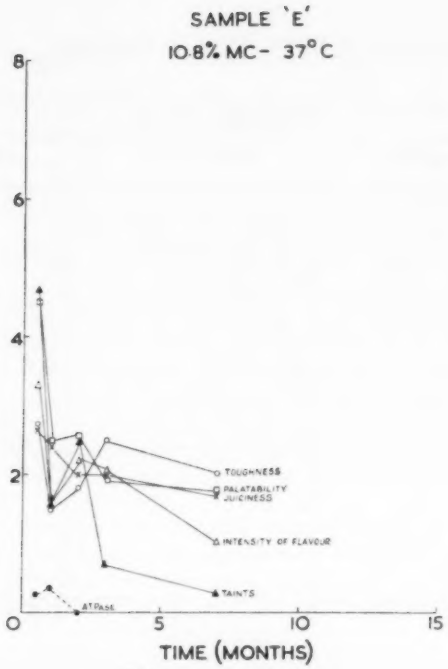


**Figure 1. Sample 'E'.**

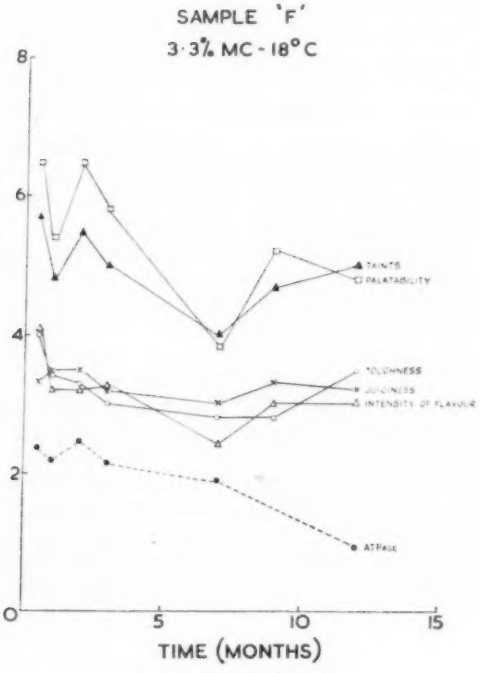


Figure 1. Sample 'F'.

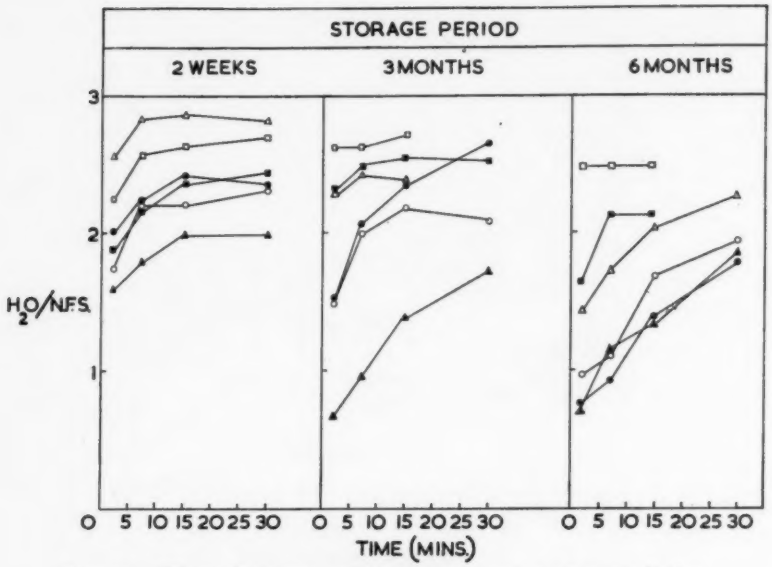


**Figure 2. Effect of storage on the ease and extent of reconstitution.**

**KEY**

A = ○ 3%, 37°C.
B = ● in-can desiccant, 37°C.
C = △ 3%, − 20°C.
D = ▲ 7%, 37°C.
E = □ 11%, 37°C.
F = ■ 3%, 18°C.

## DISCUSSION

There is some evidence to support the view that during storage dehydrated beef becomes tough and dry *(2)*. In the storage test described, however, the dryness and toughness of the samples did not increase appreciably. This may have been due to insensitivity of the panel to these attributes or to reluctance to use the extreme parts of the scale. However, Sharp *(8)* also reports that dehydrated beef does not change much in texture during storage.

It will be observed that most of these taste panel scores tend to increase slightly towards the end of the storage period. This may be due to the mood of the panel at that particular period or can be explained by the fact that there is actually very little further deterioration taking place after about 7 months. After this period the slight roast taint is quite palatable when compared with that of samples stored at a high moisture content where it is very marked and a bitter taste results.

There is considerable irregularity in the taste panel scores and it is possible only to interpret trends. The general tendency is for the panel scores to follow the decline in ATPase activity which is steady during the storage period. Measurement of its activity, or that of some other suitable enzyme in stored, dehydrated beef, might therefore prove to be a good index of deteriora-

tion, in which case such measurement could supplement or even replace assessments by a taste panel.

If one examines the taste panel scores for the whole storage period, certain facts emerge. The loss of beef flavour during storage was more marked than the change in texture, though it is difficult to say whether this was a true loss in flavour or a masking by the taints which developed. This development of taints seemed to be the major organoleptic change which occurred, and was very clearly related to the moisture content of the sample in the range of moisture levels used. The type of flavour produced in the samples with high moisture content was characteristic of non-enzymic browning reactions and these appeared to be the chief cause of deterioration.

The panel palatability ("acceptability" on the hedonic scale) marks are of interest since they followed the same pattern as those for development of taints. This suggests that the members were influenced predominantly by the taste of the sample in the absence of texture changes in assessing its palatability.

Results for ATPase assay show a decrease that is similar to the trend in all organoleptic assessments except those for toughness and juiciness. This was quite unexpected as it was thought originally that damage to the actomyosin would destroy the ATPase activity and simultaneously produce toughness and dryness. The apparent conclusion is that damage to the actomyosin sufficient to destroy its ATPase activity does not produce much toughening although the samples with high moisture content do become a little less juicy.

The good agreement with other attributes is easier to explain. Maillard reactions are the most probable origin of taints and hence of loss of flavour and palatability. In these reactions a reducing substance such as a sugar combines with a free amino group which may be part of a protein. The protein is changed; for example, it may no longer be soluble *(5, 6)* and such alterations could result in a loss of ATPase activity, and the changes observed in the organoleptic properties.

The retention of contractility in the sample stored for 16 months at either —20° C. or 18° C. but not in any of those at 37° C., is of particular interest. From the graphs it can be deduced that the ATPase activity of samples A and B at 37° C. would be expected to reach zero before 16 months, while samples C at —20° C. and F at 18° C. would probably still retain some activity after that time. This suggests that the loss of contractility and of ATPase activity are closely associated, both probably depending on denaturation of actomyosin.

The in-can desiccant in sample B reduced the moisture content to less than 1% in 3 months. Thereafter there was apparently a slight rise. This effect is probably due to absorption of water vapour from the air during the grinding of these very dry, hygroscopic samples preparatory to moisture determination. The in-can desiccant is obviously effective in removing moisture from beef and it might be advantageous to finish dehydration in the AFD process at an earlier stage and rely on storage with an in-can desiccant to dry the sample further *(2)*. This would reduce processing costs and might possibly give a better product since the material would spend less time at high temperature.

In a previous paper *(4)* it was suggested that the action of enzymes in the

dried product causes dehydrated raw meat to deteriorate more rapidly than precooked meat. The enzymes might cause deterioration directly, or indirectly by supplying reactants for the browning reaction. One requirement for the acceptance of this mechanism is that enzymes be shown to survive under conditions in which dried meat deteriorates. This work shows that ATPase is quite resistant to damage in the 'dry' state. Catalase, which is probably a more typical enzyme in respect of stability, is only inactivated after the beef containing it has suffered gross damage. It would seem desirable to carry out a further experiment in which all taste panel conditions would be carefully controlled in order to give a quantitative mathematical relationship between the subjective assessment of the panel and the objective measurements of the ATPase activity. In this way measurement of the activity of ATPase or of some other suitable enzyme in stored dehydrated beef might therefore prove to be a good index of deterioration, in which case measurement could supplement or even replace assessments by a taste panel in some circumstances.

## SUMMARY

Dehydrated raw beef of varying moisture content has been stored in an inert atmosphere at different temperatures. At intervals the reconstitution characteristics, the adenosine triphosphatase activity and the organoleptic qualities of the samples were assessed. It was found that taste panel assessments showed a deterioration in the meat which closely followed the loss of ATPase activity. The rate of deterioration of beef increased with increasing moisture content as might have been expected. Muscle fibres from samples were still contractile in the presence of adenosine triphosphate after 16 months; this confirms that relatively undamaged actomyosin was present. Catalase activity was also demonstrated in all but one of the samples after 16 months storage, suggesting that enzymes are not readily destroyed during storage. The practical and theoretical implications of these results are discussed.

### Acknowledgments

We wish to thank Mrs. M. I. Cox and her staff for carrying out the fat and moisture determinations and reconstitution tests and Miss A. M. Brown and her staff for the organization of taste panels. We are also indebted to Mr. J. R. Bendall of the Low Temperature Research Station, Cambridge, for his suggestion that contractility might be a useful guide to the behaviour of actomyosin.

## LITERATURE CITED

1. Gooding, E. G. B. and Rolfe, E. J. The vacuum contact-plate dehydration of foodstuffs. I. A first appraisal. *J. Sci. Food Agr.*, **6**, 427 (1955).
2. Harper, J. C. and Tappel, A. L. Freeze-Drying of Food Products. *Advances in Food Research*, **7**, 171 (1957).
3. Hay, J. M. The vacuum contact-plate dehydration of foodstuffs. II. Equipment. *J. Sci. Food Agr.*, **6**, 433 (1955).
4. Hunt, Sheila, M. V. and Matheson, N. A. The effects of dehydration on actomyosin in fish and beef muscle. *Food Technol.* **12**, 410 (1958).
5. Lea, C. H. and Hannan, R. S. Reactions between proteins and reducing sugars in the dry state. II. Nature of the protein groups reacting. *Biochim. biophys. Acta.*, **5**, 433 (1950).
6. Lea, C. H. and Hannan, R. S. Reactions between proteins and reducing sugars in the 'dry' state. IV. Decomposition of the amino-sugar complex and the reaction of acetylated casein with glucose. *Biochim. biophys. Acta.*, **7**, 366 (1951).
7. Rolfe, E. J. An improved method for dehydrating meat. *Food*, **25**, 199 (1956).
8. Sharp, J. G. Dehydrated Meat. DSIR Food Investigation Special Report, No. 57, H.M.S.O. 1953.

# ACCEPTANCE MEASUREMENT OF UNUSUAL FOODS*

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(Manuscript received May 31, 1958)

Customarily, alternative formulations of a food product are evaluated by consumers in terms of a frame of reference *(12)* commonly shared within the market. This frame of reference includes a set of expectations (i.e., a product image) of what the basic characteristics of the product should be, coupled with estimates of the appropriateness or utility of alternative product characteristics relative to the individual's needs in his typical or normal consumption situation *(4, 13)*. In addition, there is a possibility *(11, 15)* that biogenetically shaped preferences for certain food sensations contribute to the basis of acceptability of food products. That this latter judgmental basis is not always dominant, however, can be seen in the acceptance of the "normal" smell of Limburger cheese or of the customary bitterness of coffee. These frame-of-reference considerations of product image or product utility are not necessarily rationally and coldly applied by a judge of the acceptability of a food *(6)*. Through shared cultural training *(8)* and long personal eating experience, persons giving estimates of the acceptability of food alternatives have well-developed criteria based on normality assumptions so tacit and implicit that they may be beneath awareness.

The circumstances shaping judgments of unusual foods designed for special feeding situations can be quite otherwise, however. By definition, the needs such foods are designed to gratify are at least partly non-normal, so that normality assumptions about product utilization cannot apply. Secondly, there is no "normal" product image with which such novel formulations can be compared. It follows that when subjects lack judgmental norms on which to base evaluations of unusual foods, they may tend to put greater emphasis on the immediate sensory gratification qualities of these products. Insofar as alternative formulations of an unusual food differ in sensory attributes, these differences may be more sharply reflected in acceptability ratings than when such formulations can be judged in terms of a developed frame-of-reference including product image and utility concepts. Further, the absence of a general product image or utility concept will require that other, more remote, associations come into play, the nature of which will influence acceptance judgments. The more idiosyncratic nature of such personal associations should lead to added inter-judge variation of acceptability judgments. Some of these associations may be very far-fetched, but in an unstructured situation subjects will tend to grasp at any clue, appropriate or otherwise, which can serve as an anchor for judgment *(7)*.

The problem of evaluation of unusual foods is made additionally complex by the tendency of subjects to reject such products merely on the basis of strangeness. Recent reports by Hudson *(5)* and Torrance*(14)* confirm this

* Presented at the Eighteenth Annual Meeting of the Institute of Food Technologists, Chicago, May 27, 1958.

tendency for rejection and suspicion leading to a negative approach in the evaluation of unusual foods. It follows that if strangeness of unusual samples were decreased through provision of a utilitarian basis for judgment, negativity of acceptance ratings might be reduced, judges should be more in agreement in terms of their general level of ratings, and physical differences in alternative formulations should be less important as determinants of ratings.

## FOODS FOR IN-FLIGHT FEEDING: ACCEPTANCE TESTS AND RESULTS

An opportunity to examine conclusions about preference for strange, unusual or exotic foods with specific functional applicability arose from development of mixtures designed for in-flight feeding. Long-range high altitude supersonic aircraft require flight personnel to be encased in a restrictive pressure suit and a tightly enclosed helmet. In some instances only one hand may be available for eating during a flight extending through several meal cycles. In 1945 it was generalized that: a) acceptability of food differs on the ground and aloft; b) foods are regarded more critically in the air than on the ground, and c) some vegetables and salads are relatively less acceptable in the air than on the ground, whereas soups, meat, fruits and some vegetables are comparable and baked goods have greater relative acceptability in the air *(3)*. These generalizations were subsequently confirmed in field tests. Further, recent studies *(2)* have shown that reduced barometric pressure and the breathing of 100% aviator's oxygen do not affect the threshold of taste perception or the level of taste identification of the four primary tastes; thus the difference in level of perception and identification do not account for the differences in acceptability of food in the air and on the ground.

Within this situational, sensory and attitudinal context, some solid foods (such as bite-size sandwiches and tablets), semi-solid and liquid foods have been developed. The semi-solid food was designed to be squeezed from a tube like toothpaste and the liquid food is to be sucked from a straw like a thick malted milk. Both the latter foods are cooked meats in which particle size and fluid content have been adapted to produce desired viscosity. Field tests on the different designs indicated that certain of the bite-size sandwiches were well-liked *(1)*, that food in tablet form was poorly received *(9)*, and that liquid foods were received with mixed feelings *(10)*. Results of tests of semi-solid foods are not yet available.

To improve formulation of the liquid foods, a number of processing factors were incorporated into a factorial design for evaluation in the sensory evaluation laboratory. The first was a meat-base variable at 2 levels, chicken and ham. The chicken samples differed in terms of a second variable: suspension of meat particles by mechanical or chemical means. Ham samples varied in terms of 2 levels of saltiness. Thus there were 4 physically different samples, 2 of ham and 2 of chicken, on which preference information was required.

**Procedure.** At this point consideration was given to the question raised earlier: How meaningful are preference estimates made in the absence of a frame of reference? What could be considered the "normal" conditions of use of these in-flight samples which could serve as a basis for intercomparison of the alternative formulations? Since no comparison could be made within the "cake of custom", it was decided to vary several of the characteristics in which the field situation was estimated to differ from the normal laboratory situation. Therefore three situational factors were varied in the experiment. Some subjects received the samples hot, while some received them in the cold or lukewarm state that might be encountered in flight; some received samples in a cup, others sipped them through a tube from an opaque container as they would be consumed in flight. For some they were described only as "experimental samples," while for others they were described as "samples of Air Force meat-flavored liquid foods developed for in-flight feeding of jet pilots." These 3 variables — serving temperature, serving vessel and sample description — were incorporated into the test design in addition to the 4 variations in liquid meal composition. Ninety-six subjects drawn from male and female military and civilian personnel available at the U. S. Army Chicago Administration Center took part in this test. The experiment extended over 3 test

sessions on successive mornings; 32 subjects took part in each test session. No subjects took part in more than one test session. Samples were served in taste-test booths under controlled experimental conditions permitting no intercommunication between subjects. As each group of 4 subjects entered the laboratory they were all assigned to one of the 8 different conditions at a given session under which the 4 liquid meal formulations were to be evaluated — i.e., in cup or opaque flask; at 2 temperatures of the 3 temperature levels, and functionally described or not described. Samples were served one-by-one, and each was rated successively on the 9-point hedonic scale with rinsing taking place during a 45-second interval between successive samples. Subjects were encouraged to make comments about the samples in terms of their bases for evaluation. After completion of the preference rating task, subjects removed to an adjacent room and were interviewed by staff psychologists about their perceptions of the product. On completion of the interview subjects were requested not to discuss the experiment with anyone.

**Methodology appraisal.** Methodological hypotheses for this test centered mainly on the effect of "instruction" and "container" variables on subjects' judgments. Serving samples from opaque plastic bottles through glass straws rather than from cups was expected to increase the strangeness of the samples and the unsureness of subjects as to what the samples were; accordingly ratings were expected to be lower in this treatment group than when samples were served from china cups. The brief explanatory statement was expected to decrease strangeness of samples and thus permit higher ratings. The explanatory statement, which was typed on a card handed to each subject as he entered the taste-test booth, included a phrase on the function or utility of the foods which was supposed to give utility value of "need-satisfying" character to the product and at the same time not create false anticipation. Because this phrase made feeding from bottles meaningful, it was expected that the explanatory statement would be most effective in inducing higher ratings under the bottle condition of serving. Also, insofar as lower ratings arise from subjects' confusion or lack of frame of reference, a corresponding increase in variability of ratings could be expected.

Results of the test showed significant flavor and temperature effects, with ham samples being preferred to chicken and the hot samples being preferred to the cooler samples; differences in saltiness of ham or suspension of chicken did not affect ratings significantly.

**TABLE 1**

**Overall mean preference ratings (N = 24) assigned to samples of liquid meats rated under varying conditions**

| | *Not informed* | *Informed* | *Aver.* |
|---|---|---|---|
| Cup | 4.7 | 5.8 | 5.3 |
| Bottle | 5.4 | 5.9 | 5.7 |
| Aver. | 5.1 | 5.9 | 5.5 |

**TABLE 2**

**Variance analysis of preference ratings assigned samples of liquid meats**

| | df | SS | MS | F | Sign. |
|---|---|---|---|---|---|
| Instruction | 1 | 13.8776 | 13.88 | 23.13 | 0.1% |
| Vessel | 1 | 3.4126 | 3.41 | 5.68 | 5% |
| Instr. x Vessel | 1 | 2.7676 | 2.77 | 4.62 | 5% |
| Other Effects & Interactions | | | | | |
| Temperature | 2 | 7.0277 | 3.51 | 5.85 | 1% |
| Chicken vs. Ham | 1 | 9.8176 | 9.82 | 16.37 | 0.1% |
| Other components [1] | 43 | 51.6188 | 1.20 | 2.0 | |
| Error | 46 | 27.7630 | .60 | | |
| Total | 95 | 116.2849 | | | |

[1] These include other main effects, higher-order interactions, and judge-group (replicate) variation.

Methodological expectations regarding effects of serving vessel (cup, or bottle with tube) and instruction on ratings were only partly confirmed, however (see Tables 1 and 2). The prediction that samples would be more favorably rated by instructed subjects was supported; the difference between overall mean ratings of instructed and uninstructed groups was in the expected direction and significant at the 5% level. However, predictions on the main effects of serving vessel, and on the interaction between instruction and serving vessel, were not confirmed. Subjects receiving samples from opaque bottle with glass straw rated samples significantly (0.1%) higher than subjects receiving samples from cups; correspondingly, the effects of instruction were most marked among the cup subjects (interaction significant at the 5% level).

Comments of subjects on what they liked and disliked about the samples (Tables 3 and 4) showed that while both instructed and uninstructed groups favored the taste and flavor

**TABLE 3**

**Comments of subjects on what they liked about the liquid meat samples**

| Comments | Cup | | Bottle | | Total |
|---|---|---|---|---|---|
| | Instructed | Not instructed | Instructed | Not instructed | |
| Taste and flavor | 13 | 10 | 15 | 17 | 55 |
| Concept of meat in liquid form | 6 | 2 | 5 | 1 | 14 |
| Tickness and richness | 3 | 3 | 6 | 1 | 13 |
| All others and general | 4 | 4 | 4 | 1 | 13 |
| Nothing | 5 | 4 | 2 | 3 | 14 |
| No answer | 2 | 3 | 1 | 2 | 8 |
| Total [1] | 32 | 27 | 33 | 25 | 117 |

[1] Responses total more than 24 because of multiple answers.

of the samples, the uninstructed group was "put-off" by the strangeness of the samples, and the instructed group was impressed by the idea of liquidized meat as an approach to in-flight feeding. This utilitarian aspect of the product reflected by the data might account for the favorable increment in ratings from instructed subjects. Insofar as this concept applied equally to responses toward different samples it might be expected that the instructed group would be less sensitive to formulation and flavor differences between the samples. A breakdown of average variation within cells of subjects-groups (Table 5) shows this may be true: the ratio of samples-plus-sample-cell interaction variance between the two instructional groups is significant, with ratings of the instructed group less variant in terms of treatment differences and interactions. Further, the uninstructed group was more variant also in terms of inter-subject differences in level of rating of liquid meat samples and in evaluations as to which samples were how much preferred to others (sample-subject interaction).

It was expected that the unfamiliar method of presentation of samples in bottles with glass straws would adversely affect level of ratings, but the opposite occurred. It may be that sipping samples from bottles implied to uninstructed subjects the novelty of the liquid meals known to the instructed subjects, and this novelty attracted the interest of subjects. Alternatively, it may be that the color, odor, temperature or other disliked qualities of the samples were less apparent when samples were drawn out of opaque bottles, so that relatively more weight was given to flavor as a determinant of ratings; as indicated in Table 3, flavor of the samples was generally liked. The cup subjects offered considerably more critical comments on the samples (Table 4), particularly about the coldness of the samples, their thickness and their chalky texture; further, the fact of more criticisms on

**TABLE 4**

**Comments of subjects on what they disliked about the liquid meat samples**

| Comments | Cup | | Bottle | |
|---|---|---|---|---|
| | Instructed | Not instructed | Instructed | Not instructed |
| Too cold | 11 | 12 | 4 | 9 |
| Too thick | 6 | 10 | 6 | 4 |
| Chalky texture | 7 | 7 | 5 | 4 |
| Too salty & spice | 5 | 7 | 8 | 2 |
| Flavor & Odor | 1 | 5 | 1 | 3 |
| Concept of meat in liquid form | 3 | 2 | 5 | 6 |
| Richness, fattiness, greasiness | 5 | 5 | 5 | 4 |
| Appearance, color | 5 | 3 | 1 | 2 |
| Strangeness | — | 5 | — | 6 |
| All others | — | — | 3 | — |
| Nothing | 2 | — | — | 1 |
| No answer | 1 | 2 | 2 | — |
| Total [1] | 46 | 58 | 40 | 41 |

[1] Responses total more than 24 because of multiple answers.

*appearance* from cup subjects supports the supposition that bottle subjects were less aware of product characteristics.

### A Followup Test

In a later sensory evaluation of two alternative formulations of liquidized meat, subjects (N = 40) were informed as to the nature of the product and received the samples *both* in cups and in bottles. Samples were rated singly and one-by-one in the hedonic scale. Half the subjects received the 2 formulations in cups, followed by the same samples in the opaque bottles with glass straws, while the other half received the 2 samples first in bottles and then in cups. Samples were served at lukewarm temperature only.

On the basis of the previous test reported above, it was expected that subjective preference differentiation between alternative formulations would be reduced when samples were taken from the opaque bottles, and that insofar as this was true, the ratings of samples in bottles would tend to be higher. Neither of these expectations was wholly supported (Table 6). The bottle group provided ratings no higher than the cup group, and there was equivalent differentiation between the two formulations in both serving vessels.

However, whereas the "cup-first" group indicated in their comments (Table 7) that they were very much aware of the odd textural qualities (thick, gritty, chalky, dry) of the products and that they differentiated between the samples on this dimension even when samples were later served from a bottle, this was not true of the "bottle-first" group; the latter "bottle-first" group did not use texture as a basis for differentiation even when samples were subsequently provided in cups. Further, it is interesting to note that the "bottle-first" group commented on the appearance of samples later served in cups, suggesting that this dimension was not drawn to their attention previously. Other differences in dimensional bases for comments also appeared, and generally the "cup-first" group was more prolific in comments.

These observations on the way subjects perceived and differentiated the products on the basis of initial introduction to them are supported by the finding the interaction of the product with vessel-used-first was significant at the 10% level. It would appear that when liquid meat samples in bottles with glass tubes are introduced to subjects, unfavorable textural and other qualities are less important as pertinent dimensions in preference evaluation. When samples are first introduced to subjects in cups, however, judgments based on texture, flavor, etc., are *also* applied when samples are later served in bottles.

**TABLE 5**

**Components of variation of ratings obtained under different instructions and serving vessel conditions [1]**

| | Not instructed | | | | Instructed | | | | Both Instructions | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | af | SS | MS | F | af | SS | MS | F | af | SS | MS | F |
| **CUPS** | | | | | | | | | | | | |
| Subjects w/in cells [1] | 18 | 282.13 | 15.67 | | 18 | 208.26 | 11.57 | | 36 | 490.39 | 13.62 | |
| Samples & sample-cell [1] interactions | 18 | 89.63 | 4.98 | 2.12 | 18 | 20.25 | 1.12 | .82 | 36 | 109.88 | 3.05 | 1.15 |
| Subject-sample and subject-sample-cell [1] interactions | 54 | 126.87 | 2.35 | | 54 | 73.24 | 1.36 | | 108 | 200.11 | 1.85 | |
| Sum | 90 | 498.63 | 5.54 | | 90 | 301.75 | 3.35 | | 180 | 800.38 | 4.45 | |
| **BOTTLES** | | | | | | | | | | | | |
| Subject w/in cells [1] | 18 | 219.70 | 12.20 | | 18 | 135.50 | 7.53 | | 36 | 355.20 | 9.87 | |
| Samples & sample-cell [1] interactions | 18 | 53.70 | 2.98 | 1.51 | 18 | 26.50 | 1.47 | .75 | 36 | 80.20 | 2.23 | 1.14 |
| Subject-sample and subject-sample-cell [1] interactions | 54 | 106.55 | 1.97 | | 54 | 104.50 | 1.95 | | 108 | 211.05 | 1.95 | |
| Sum | 90 | 379.95 | 4.22 | | 90 | 266.50 | 2.96 | | 180 | 646.45 | 3.59 | |
| **BOTH VESSELS** | | | | | | | | | | | | |
| Subject w/in cells [1] | 36 | 501.83 | 13.94 | | 36 | 343.76 | 9.54 | | 72 | 845.59 | 11.74 | |
| Sample & sample-cell [1] interactions | 36 | 143.33 | 3.98 | 1.84 | 36 | 46.75 | 1.29 | .78 | 72 | 190.08 | 2.64 | 1.39 |
| Subject-sample and subject-sample-cell [1] interactions | 108 | 233.42 | 2.16 | | 108 | 177.74 | 1.65 | | 216 | 411.16 | 1.90 | |
| Sum | 180 | 878.58 | 4.88 | | 180 | 568.25 | 3.16 | | 360 | 1446.83 | 4.02 | |

[1] Since a total of 96 subjects took part in this test, it follows that 24 subjects evaluated the four samples in each of the four conditions of "instruction" and "vessel"; these 24 subjects were grouped into six cell-groups, each with N = 4, receiving samples under different temperature and replicate-session conditions.

**TABLE 6**

**Mean preference ratings (N = 20) for two formulations of liquid meats served in cups and in bottles**

| | Sequential positions 1 & 2 | | Sequential positions 3 & 4 | | Average |
|---|---|---|---|---|---|
| | Cup | Bottle | Cup | Bottle | |
| Formulation A | 5.7 | 6.2 | 6.0 | 5.9 | 6.0 |
| Formulation B | 5.0 | 5.9 | 6.1 | 5.2 | 5.6 |
| Average | 5.4 | 6.1 | 6.0 | 5.5 | 5.8 |

**TABLE 7**

**Comments made by subjects (N = 20) evaluating two formulations of liquid meat served from cups or from opaque bottles**

| Comment | Sequential positions 1 and 2 | | Sequential positions 3 and 4 | |
|---|---|---|---|---|
| | Cup | Bottle | Cup | Bottle |
| Salty, spicy, smoky, strong | 9 | 3 | 4 | 7 |
| Thick, gritty, chalky, dry | 8 | 1 | — | 5 |
| Flavor (good or poor) | 6 | 3 | 2 | 2 |
| Don't like drinking liquid meat | 1 | 2 | 1 | 1 |
| Color and appearance | — | — | 4 | — |
| Total | 24 | 9 | 11 | 15 |

## SUMMARY AND CONCLUSIONS

Strangeness of samples has been shown to lead to relative rejection of products in the comparative absence of clues to a frame of reference within which judgment may take place. Variation in clues selected by judges as a basis for evaluation lead to greater inter-judge disagreement. Addition of a functional (utilitarian) basis for judgment tends to reduce relative importance of product physical characteristics as a basis for judgment. In the absence of any judgmental frame of reference, reduction in the number of product physical attributes apparent to the judge appears to reduce operation of bases for rejection and increase homogeneity of judgment between subjects; inter-sample discrimination is also reduced. Once a basis for judgment has been established from available clues, it tends to persist even though additional product characteristics may become later available to perception. Insofar as these observations may hold in a situation in which unusual foods are used, it would appear that acceptance could be maximized if such foods could be introduced with a specific functional application and in a form or fashion in which their novel qualities would be least apparent.

## LITERATURE CITED

1. Finkelstein, Beatrice, Pippitt, Robert G., and Taylor, Albert A. Evaluation of improved compact box lunch. *WADC Technical Report 56-363,* July 1956.

2. Finkelstein, Beatrice, and Pippitt, Robert G. Effect of altitude and oxygen upon taste. *WADC Technical Report 57-261,* April 1957.

3. Green, D. M., and Butts, J. S. Factors affecting acceptability of meals served in the air. *J. Am. Dietet. Assoc.,* **21,** 415 (1945).

4. HOWELL, L. D. Consumer preference and demand research. *J. Farm Economics*, **36**, 641 (1954).
5. HUDSON, B. B. Anxiety in response to the unfamiliar. *J. Social Issues*, **10**, 53 (1954).
6. IRWIN, F. W. An analysis of the concepts of *discrimination* and *preference*. *Am. J. of Psychol.*, **71**, 152 (1958).
7. KRUCH, DAVID AND CRUTCHFIELD, RICHARD. *Theory and Problems in Social Psychology*. 1948. McGraw-Hill, New York, N. Y.
8. LEWIN, KURT. Forces behind food habits and methods of change. *National Research Council Bulletin 108*. (1945), p. 35.
9. MOCK, RONALD O., AND PIPPITT, ROBERT G. Field test of food tablets. *WADC Technical Note 56-370*. (1956).
10. MOCK, RONALD O. Acceptability test of liquid meats. *WADC Technical Note 57-63*. (1957).
11. MULLER, H. J., Human values in relation to evolution. *Science* **127**, 625 (1958).
12. NEWCOMB, THEODORE M., *Social Psychology*. 1952. Tavistock Publications, Ltd., London.
13. POLITZ, ALFRED, AND DEMING, W. EDWARDS. On the necessity of presenting consumer preferences as predictions. *J. Marketing*, **18**, 18 (1953).
14. TORRANCE, E. PAUL. Sensitization versus adaptation in preparation for emergencies: Prior experience with an emergency ration and its acceptability in a simulated survival situation. *J. Appl. Psychology*, **42**, 63. (1958).
15. YOUNG, P. T. "The role of hedonic processes in the organization of behavior," *Psychological Rev.* **59**, 249. (1952).

# INTERACTION OF SUCROSE AND CALCIUM CYCLAMATE ON PERCEIVED INTENSITY OF SWEETNESS[a,b]

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(Manuscript received August 1, 1958)

If we mix various proportions of 5 and 10% sucrose solutions, we will find that the perceived intensity of sweetness of the mixtures will lie between the perceived intensities of the original solutions. Specifically, the subjective intensities of the mixtures should be approximately geometrically proportional to the relative amounts of each of the original solutions that comprise the mixture. In this case, the effects of mixing the solutions are said to be additive.

Consider next the case where, instead of two sucrose solutions, we have one sucrose solution and one of another type of sweetening agent, calcium cyclamate. Are the effects of mixing two different sweetening agents also additive? Will the sweetness of the mixture be proportional to the amounts of each pure solution in the mixture, or will the sweetness be greater or less than of either pure solution alone?

The purpose of this study was to determine the effects of mixing, in different proportions, various concentrations of two chemically distinct sweeteners upon the perceived intensity of sweetness.

## EXPERIMENTAL

**Subjects.** The subjects (*Ss*) were 16 employees of this Institute, most of whom had participated in previous sweetness studies *(2)*. Those who had no prior experience were selected from candidates who demonstrated ability in discriminating among different sucrose concentrations.

**Method.** The method used was that of single stimulus with a nine-interval rating scale of intensity. Alternate intervals were anchored with the following descriptions of intensity: *None, slight, moderate, strong,* and *extreme.* The intervals were assigned successive integers from 1 *(none)* to 9 *(extreme)* and the ratings then treated quantitatively.

**Concentrations of sucrose and cyclamate.** The sucrose used to prepare the solutions was an analytical reagent at least 99.991% pure. The calcium cyclamate was Du Pont *Cylan.* Charcoal-filtered distilled water was used as the solvent.

Estimates of the concentrations of sucrose and cyclamate required to give mean intensity ratings of 2, 4, 6 and 8, were available from both published *(2)* and unpublished [c] data of Schutz and Pilgrim. For sucrose, these concentrations were 2.31%, 5.79%, 14.63%, and 37.07%, respectively. For cyclamate the corresponding concentrations were: .067%, .166%, .465%, and 1.540%. Note that the concentrations are approximately logarithmically spaced within each set.

**Solutions.** At each level, two solutions of mixtures of sucrose and cyclamate were

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[a] This paper reports research undertaken at the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned Number 928 in the series of paper approved for publication.

[b] The writer expresses his appreciation to Mr. Norman Gutman, Chief, Statistics Office, QMFCIAF, for his valuable assistance. Special acknowledgment is made to Mrs. Phyllis Whitmer and to Messrs. Bill Lewis and Ralph Lundgren who performed almost all the laboratory work.

[c] Personal communication.

prepared, as well as solutions of sucrose and cyclamate alone. The first mixture consisted of two-thirds of the sucrose solution and one-third of the cyclamate. In the second mixture, the proportions were reversed. For example, at Level I (estimated intensity rating of 2), there were 4 solutions: (a) 2.31% sucrose; (b) 1.54% sucrose plus .022% cyclamate; (c) .77% sucrose plus .045% cyclamate; (d) .067% cyclamate. Thus, mixture (b) consisted of two-thirds (a) and one-third (d); and mixture (c) consisted of one-third (a) and two-thirds (d).

The solutions for the other 3 levels were similarly prepared. Table 1 presents the concentrations, by level, of the solutions containing only sucrose, only cyclamate, and the mixtures.

**TABLE 1**

**Concentrations of pure solutions of sucrose and calcium cyclamate and of Their Mixtures**

| Level | Estimated subjective intensity of sweetness | Sweetener is: (a) All sucrose | | (b) ⅔ Sucrose; ⅓ Cyclamate | | (c) ⅓ Sucrose; ⅔ Cyclamate | | (d) All cyclamate | |
|---|---|---|---|---|---|---|---|---|---|
| | | Su. | Cy. | Su. | Cy. | Su. | Cy. | Su. | Cy. |
| I | 2 | 2.31% [1] | 0% | 1.54% [1] | .022% | .77% | .045% | 0% | .067% |
| II | 4 | 5.79% | 0% | 3.86% | .055% | 1.93% [1] | .111% | 0% [1] | .166% |
| III | 6 | 14.63% [1] | 0% | 9.75% [1] | .155% | 4.88% | .310% | 0% | .465% |
| IV | 8 | 37.07% | 0% | 24.71% | .510% | 12.36% [1] | 1.030% | 0% [1] | 1.540% |

[1] Denotes solutions and mixtures presented to *S*s on days 1 and 3. Other solutions and mixtures were presented on days 2 and 4.

**Procedure.** The 16 solutions (4 solutions at each of 4 levels) were divided into 2 groups of eight. On testing days 1 and 3, the 8 solutions indicated by a footnote symbol in Table 1 were presented to the *S*s; on testing days 2 and 4, the other 8 solutions were evaluated. Order of presentation of the samples was balanced. All 4 testing days were spaced approximately 2 weeks apart. Fresh solutions were prepared for each test day.

*S*s sat in a semi-enclosed testing booth. Six ml. samples in coded one-ounce glasses were presented one at a time through a turntable in a wall separating the booth from the serving area. After rating each sample, *S* rinsed his mouth *ad libitum* with charcoal-filtered distilled water. The time between the rating of one solution and the presentation of the next was 45 seconds.

## RESULTS

The mean intensity ratings for each solution over two sessions are presented in Table 2. It was estimated that the successively increasing concentrations of the sucrose solutions alone and of the cyclamate solutions alone would yield intensity ratings of 2 (Level I), 4 (Level II), 6 (Level III), and 8 (Level IV). The estimates were somewhat more accurate for the sucrose solutions than those for the cyclamate. For both types of sweeteners the set of stimulus concentrations is considered adequate to test for additivity of mixtures over a wide range of subjective intensities.

If the intensities of the mixtures possess the property of additivity, then their ratings should lie between those of the pure cyclamate and the pure sucrose solutions. Inspection

**TABLE 2**

**Mean intensity ratings for each solution and mixture**

| Level | Sweetener is: (a) All sucrose | (b) ⅔ Sucrose; ⅓ Cyclamate | (c) ⅓ Sucrose; ⅔ Cyclamate | (d) All cyclamate |
|---|---|---|---|---|
| I | 2.25 | 2.41 | 2.06 | 2.06 |
| II | 3.75 | 4.22 | 3.91 | 3.62 |
| III | 6.03 | 6.59 | 6.59 | 5.44 |
| IV | 7.69 | 7.81 | 7.66 | 7.72 |

of Table 2 suggests that at the lowest and highest stimulus concentrations (Levels I and IV), the intensity of the mixtures are not markedly different from the intensities of the pure solutions. For the intermediate levels, however, the intensities of the mixtures are always higher than those of the pure solutions.

An analysis of variance was performed to test whether the apparent departures from additivity, particularly at the second and third levels, are significant. The breakdown of the sources of variation and the results of the analysis are shown in Table 3. The ratings of the two mixtures at each intermediate level are significantly greater than the ratings of the pure sucrose and cyclamate solutions. This effect is not evident for either the highest or lowest levels.

The significance of "$L_1$" and "$L_2$" in Table 3 merely means that the intensity of sweetness increases as "linear" and "cubic" functions of stimulus concentrations. "Linear" and "cubic" are in quotation marks because the sums of squares for "$L_1$" reflects some cubic as well as linear components. Similarly, the sum of squares for "$L_2$" reflects both

**TABLE 3**

**Analysis of variance of sweetness ratings**

| Source of variation | df | Mean square | Level of significance |
|---|---|---|---|
| $T_1$ (within Level I) | 1 | 2.26 | |
| $T_1$ (within Level II) | 1 | 1.53 | |
| $T_1$ (within Level III) | 1 | 2.82 | |
| $T_1$ (within Level IV) | 1 | .12 | |
| $T_2$ (within Level I) | 1 | .20 | |
| $T_2$ (within Level II) | 1 | 4.50 | .05 |
| $T_2$ (within Level III) | 1 | 23.63 | .001 |
| $T_2$ (within Level IV) | 1 | .03 | |
| $T_1 T_2$ (within Level I) | 1 | .20 | |
| $T_1 T_2$ (within Level II) | 1 | .28 | |
| $T_1 T_2$ (within Level III) | 1 | 2.82 | |
| $T_1 T_2$ (within Level IV) | 1 | .28 | |
| $T_1$ (within Level I) — $S_2$ | 1 | .63 | |
| $T_1$ (within Level II) — $S_2$ | 1 | 1.53 | |
| $T_1$ (within Level III) — $S_2$ | 1 | .07 | |
| $T_1$ (within Level IV) — $S_2$ | 1 | .03 | |
| $T_2$ (within Level I) — $S_2$ | 1 | 1.32 | |
| $T_2$ (within Level II) — $S_2$ | 1 | 2.00 | |
| $T_2$ (within Level III) — $S_2$ | 1 | .95 | |
| $T_2$ (within Level IV) — $S_2$ | 1 | 2.00 | |
| $T_1 T_2$ (within Level I) — $S_2$ | 1 | 1.32 | |
| $T_1 T_2$ (within Level II) — $S_2$ | 1 | 2.53 | |
| $T_1 T_2$ (within Level III) — $S_2$ | 1 | .01 | |
| $T_1 T_2$ (within Level IV) — $S_2$ | 1 | 3.12 | |
| $L_1$ | 1 | 1953.12 | .001 |
| $L_2$ | 1 | 334.76 | .001 |
| $L_1 L_2$ | 1 | .50 | |
| $S_2$ | 1 | 6.57 | .05 |
| $L_1 S_2$ | 1 | 6.12 | .05 |
| $L_2 S_2$ | 1 | .63 | |
| $L_1 L_2 S_2$ | 1 | .28 | |
| Judge (within day) | 60 | 6.97 | .05 |
| Judge-treatment interaction: within day (Error) | 420 | 1.33 | |
| Total | 511 | | |

Note: Symbols refer to comparisons of mean ratings:
(See Table 1 or 2)
$T_1$ — Sweeteners (a) and (b) *vs.* (c) and (d)
$T_2$ — Sweetners (a) and (d) *vs.* (b) and (c)
$T_1T_2$ — Sweeteners (a) and (c) *vs.* (b) and (d)
$L_1$ — Levels I and II *vs.* III and IV (Linear component)
$L_2$ — Levels I and III *vs.* II and IV (Cubic component)
$L_1L_2$ — Levels I and IV *vs.* II and III (Quadratic component)
$S_2$ — Days 1 and 2 *vs.* 3 and 4

linear and cubic components. To determine the significance of the entirely linear and entirely cubic mathematical relationships, we used orthogonal polynomials as described by Fisher and Yates *(2)*. The sums of squares for the linear component increased to 2276.33 and the sums of squares for the cubic component decreased to 11.56. We may conclude that the ratings and logarithms of the stimulus concentrations are linearly related, and that the cubic component is not too important though still statistically significant at the .01 level.

The significance of "$S_2$" indicates that the ratings of intensity were higher on days 3 and 4 than on days 1 and 2. The interaction between replication and the linear component of stimulus concentration ($L_1S_2$) is also statistically reliable. It is possible that during the first 2 days *S*s were reluctant to endorse the 8th and 9th categories because, had even stronger concentrations been later presented, they would have no "room" for expressing their more intense sensations. However, once having learned the range of concentrations during the first 2 days, they would be less hesitant to use the extreme categories during the second 2 days. This explanation has no special relevance to the main conclusions of the experiment.

## DISCUSSION

A practical implication of the results is that if these two sweetening agents are both used in a product, the sweetness will probably be greater than would be expected on the basis of simple additivity. Whether the same conclusion would hold when pairs (or triplets) of other sweeteners are employed is to be determined in future investigations.

At least two alternative explanations might account for this fact of non-additivity in the middle range of concentrations. The first is that the sweet receptors may be differentially sensitive to the sucrose and cyclamate molecules. If they are, then a mixture of the two sweeteners would be expected to stimulate a greater number of receptors, and consequently, to bring about a greater perceived intensity.

The alternative explanation assumes that the presence of bitter reduces the perceived intensity of sweet. Several *S*s and the experimenter himself observed that many of the solutions which contained cyclamate were somewhat bitter. If these observations are valid, then the mixtures of sucrose and cyclamate could be expected to taste sweeter than the pure solutions because the bitter component of the cyclamate is diluted in the mixture and hence suppresses the sweet taste to a lesser degree. A further extension of this reasoning is: If two sweetening agents have no bitter component, then the effects of mixing them are more likely to be additive than if either or both are somewhat bitter. The implications of this explanation can be subjected to experimental test.

## SUMMARY

Pure solutions of two sweetening agents, calcium cyclamate and sucrose, and mixtures of these solutions were rated by judges for intensity of sweetness. At the highest and lowest solution concentrations, the perceived intensities of the mixtures did not depart markedly from the perceived intensities of the average of the pure solutions comprising the mixtures. At moderate solution concentrations, however, the perceived intensities of the mixtures were significantly higher than the intensities of the pure solutions. The practical and experimental implications of this finding were discussed.

## LITERATURE CITED

1. Fisher, R. A., and Yates, F. *Statistical Tables for Biological, Agricultural and Medical Research.* 1948. Hafner, New York, N. Y.
2. Schutz, H. G., and Pilgrim, F. J. Sweetness of various compounds and its measurement. *Food Research*, **22**, 206 (1957).

# CARBONYLS IN OXIDIZING FAT. I. SEPARATION OF STEAM VOLATILE MONOCARBONYLS INTO CLASSES[a]

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Carbonyl compounds influence the flavor and quality of many foods and natural products. They are believed to be largely responsible for the rancid flavors developed in oxidizing fat and fatty tissue. However, relatively little is known concerning the influence of composition, treatments, and conditions on the kinds and amounts of carbonyls formed in fat, and their relationship to odor and flavor *(11, 18, 23, 30, 38)*. The objectives of this work were to determine some of the changes occurring in fractions of the steam volatile carbonyls of freezer-stored pork fat.

There is considerable need for a more specific objective method of determining rancidity. The shortcomings of the organoleptic methods *(1, 2, 21, 34, 35)* have been fully described elsewhere *(4, 24, 36, 47)*. Peroxide values can be used to follow the amount of oxidation, but they do not constitute a specific determination of rancidity *(31, 32, 33, 36, 38)*. Rancidity has been found to appear at different peroxide levels. Carbonyls are produced as a result of the decomposition of the peroxides, and apparently their nature depends on the kind of hydroperoxides, conditions, and other variables *(38)*.

In recent years the sensitivity of methods involving 2, 4-dinitro-phenylhydrazone[b] derivatives of carbonyls has been applied. Pool and Klose *(40)* have devised a method for determining total monocarbonyls, Neumer and Dugan *(37)* have reported a procedure for total volatile carbonyls, and Chang and Kummerow *(10)* a method for total and volatile carbonyls. In general, applications of the above methods have indicated a close relationship between peroxides and carbonyl values. Total carbonyl values, therefore, also appear not to be specific for rancidity.

Consideration of these facts, together with the well-known sensitivity of taste, leads to the assumption that rancid flavors may be due to some particular type of carbonyls *(8, 9, 14, 15, 27)*, the proportions and rate of formation of which are influenced by the composition of the glycerides, catalysts, enzymes, and anti-oxidants present, and processing and storage conditions. Henick, Benca, and Mitchell *(23)* have developed a method for determination of total saturated and unsaturated carbonyls as hexanal and crotonaldehyde. Applications *(23, 38)* of this method showed the rate of increase of saturated carbonyls exceeded that of the 2-enal class. The method does not take into account other classes of carbonyls that may be present.

In studies of autoxidative mechanisms *(6, 8, 9, 22, 25, 27, 28, 44, 45, 46)* one or more compounds of the following classes have been identified: saturated aliphatic aldehydes, saturated ketones, 2-en-1-als, 2, 4-dienals, and dicarbonyls.

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[a] Presented at the Eighteenth Annual Meeting of the Institute of Food Technologists, Chicago, Ill., May 27, 1958.

[b] Hereinafter referred to as DNPH.

A number of investigators have recently reported identification of carbonyls in food products *(12, 14, 15, 39, 48)*. A quantity of individual compounds of the expected types of carbonyls were found by Forss, Pont, and Stark *(14, 15)* in off-flavored skim milk, and by Pippen, Nonaka, Jones and Stitt *(39)* in a simmering mixture of chicken muscle tissue and water. For the most part, carbonyls were isolated from large amounts of material, and DNPH derivatives obtained in large enough quantity to permit separation by repeated column adsorption chromatography, and identification by conventional procedures. Such methods are laborious and time-consuming and are not suited to the following of qualitative and quantitative changes during oxidation.

This paper describes the application of procedures for the separation and determination of total volatile carbonyls, monocarbonyls, and dicarbonyls of pork fat. The volatile monocarbonyls were separated into classes by a rapid new paper chromatographic procedure *(19)* for separation of mixtures of 2-alkanone, alkanal, alk-2-enal, and alk-2,4-dienal DNPH derivatives. These procedures were referred to in a recent note *(18)* which described the identification of volatile saturated aliphatic aldehydes and methyl ketones in a rancid pork fat.

The new method for separation of homologous series of 2-alkanones, alkanals, alk-2-enals, and alk-2,4-dienals into classes described by Gaddis and Ellis *(19)* fails only with respect to acetone, methanol, and ethanal. Acetone separates in the alkanal class, and methanol and ethanal come out in the alk-2-enal class *(19)*. As a result, when the classes are separated into individual compounds *(13)*, mixtures of ethanal-acrolein, acetone-propanal, and acetone-butanal are obtained *(19)*. However, the presence of acetone, methanol, and ethanal in the mixture of monocarbonyl DNPH's can be detected by the partial extraction of these compounds from carbon tetrachloride with a 2 N HCl solution of 2,4-dinitrophenylhydrazine *(18)*. Spectrophotometric studies in neutral solution and in alcoholic alkali *(13, 26)* are useful in the detection of such mixtures, and where necessary corrections can be applied. Over-all, the methods of Ellis, Gaddis, and Currie *(13)* and Gaddis and Ellis *(19)* result in a powerful tool for rapid identification and estimation of classes and individual compounds. In the freezer-stored pork fat *(18)* examined so far, acrolein has not been detected, and methanol, ethanal, acetone, and methyl ethyl ketone are usually present only in trace amounts. These compounds are in part removed from the other monocarbonyls by the extraction procedure. Hexanal and propanal were identified as the major saturated compounds.

## EXPERIMENTAL

**Solvents and reagents.** A. C. S. grades of carbon tetrachloride, benzene, methanol, and commercial absolute ethanol, Skellysolve C[e] (b 91° —95°) were purified as described by Ellis, Gaddis, and Currie *(13)*. Technical grade petroleum ether was distilled and the fraction boiling 37° —40° C.

[e] Mention of specific commercial materials or equipment throughout this paper does not constitute recommendation for their use above similar materials and equipment of equal value.

was collected for use. Alcoholic potassium hydroxide (0.25 N) was prepared as described by Ellis, Gaddis, and Currie *(13)*. Tetrahydrofuran was redistilled. A. C. S. grade anhydrous ether; propylene glycol U. S. P.; and vaseline (white petroleum jelly) Blue seal, Cheesbrough Manufacturing Company, were used without further treatment. The 2,4-dinitrophenylhydrazine reagent was prepared by grinding the 2,4-dinitrophenylhydrazine [d] in a mortar with 2N HCl and filtering. This gave a solution containing approximately 5 mg. DNP per ml. The solution was purified by shaking with carbon tetrachloride in a ratio of 1 to 5, separating and filtering *(3)*. This reagent was prepared fresh daily.

**Materials and equipment.** Fat samples were steam distilled in detachable 32 x 300 mm. culture tubes connected to a micro Kjeldahl-type steam distillation apparatus. Alcoa alumina (grade F-20) was modified according to Pool and Klose *(40)* by mixing with 20% of hydrated alumina. Whatman No. 3 filter paper sheets were cut in tapering strips as described by Ellis, Gaddis, and Currie *(13)*. Similarly, 38 × 300 mm. culture tube chromatographic chambers, with pressure regulation by mercury traps, were employed *(13)* in ascending solvent development. Spectrophotometric measurements were made with a Beckman Model DU, using a tungsten lamp as light source.

**Procedure.** Ground fat tissue was rendered at as low a temperature as possible on the steam bath. As fast as formed, the melted fat was filtered through cheesecloth and 10.0 g. portions weighed into 32 × 300 mm. culture tubes. Two determinations were usually made on each sample of tissue fat — on the rendered fat, and on the rendered fat after heating at 165° C. for 15 minutes to approximate the effect of cooking. Carbonyls were volatilized by steam for 22 minutes at a distilling rate of 6 ml. per minute. The distillate was collected in an ice-cooled flask containing 50 ml. of 2N HCl solution of DNP, composed of 10 to 50 ml. of saturated DNP reagent. During this operation, the condenser tip was immersed in the reagent, and, when the steam distillation was completed, the tip was washed inside and out with carbon tetrachloride. In some experiments, carbonyls which volatilized during the heating (cooking) process at 165° C. were collected and reacted with the DNP reagent in a similar manner. The steam distillate reaction mixtures were allowed to stand overnight at room temperature. The cloudy solution, or suspension, of DNPH'S was extracted with carbon tetrachloride and then benzene. In this operation both extracts were suitably washed with 2N HCl and then water. The extracts were dried over powdered anhydrous sodium sulfate and filtered, and, when total carbonyls were measured, the carbon tetrachloride extracts were made to 100 ml. Solvent was removed from the benzene extracts on the steam bath with a jet of nitrogen gas, and residues made to a suitable volume with carbon tetrachloride. Determination of total carbonyls were made with the spectrophotometer by measurement of the absorbance at the maxima.

The total carbonyls were separated into monocarbonyls and dicarbonyls, and freed of excess DNP by chromatography on 0.35" × 2.5" columns of 20% hydrated alumina. Solvent was removed from the two fractions on a steam bath with a jet of nitrogen gas. The two residues were dissolved in minimum quantities of carbon tetrachloride and applied to separate columns of alumina, prepared by adding about ¼ in. of powdered anhydrous sodium sulfate and passing through a preliminary portion of benzene. The monocarbonyls were separated and eluted with benzene. After development with ether, the dicarbonyls were eluted with ethanal. Solvents were removed from the monocarbonyl and dicarbonyl fractions and the residues dissolved in carbon tetrachloride and made to a convenient volume. The absorbance at the wave length of maximum absorption was determined for each fraction.

The volatile monocarbonyls were resolved into classes by a rapid paper chromatographic procedure *(19)*. Mechanically, this technique was the same as that described by Ellis,

[d] Hereinafter referred to as DNP.

Gaddis, and Currie *(13)*. In order to compare fractions, on the basis of proportions of classes, the same quantities of total coloring components were spotted. Amounts spotted were calculated by the following formula:

$$\text{X ml. of carbonyl DNPH solution} = \frac{1.5 \text{ (monocarbonyls)}}{\text{absorbance of carbonyl DNPH solution}}$$

The aliquot thus determined was evaporated on the steam bath with a jet of nitrogen gas to a suitable volume and spotted on the paper. Each separation was run in groups of 3 strips or more in order to obtain enough material for characterization of the separated spots by spectral analysis. Extracted material was dissolved in 3.00 ml. carbon tetrachloride and the absorbance determined. Occasional determinations were also made in alcoholic alkali. This was usually done by removal of the solvent from the sample read in neutral solution, adding 0.30 ml. of benzene and 2.70 ml. of alcoholic potassium hydroxide, mixing and reading immediately. Preliminary paper chromatographic separation of the dicarbonyl fraction was also made, using a similar technique. Paper chromatograms were run at room temperature, using a mixture of 5.00 ml. of Skellysolve C and 1.00 ml. of tetrahydrofuran as ascending solvent. This solvent combination was suggested by Rice, Kellar, and Kirchner *(41)*.

Paper chromatographic separation of the classes of monocarbonyls into their individual compounds was made by the method of Ellis, Gaddis, and Currie *(13)* and Gaddis and Ellis *(19)*.

Peroxides were determined on the rendered fat by the method of Rockwood, Ramsbottom, and Mehlensbacker *(42)*.

## RESULTS AND DISCUSSION

**Carbonyl values.** Total carbonyl, and mono- and dicarbonyl values, were determined for a considerable quantity of freezer-stored cured and uncured pork tissue samples from different animals. Statistical analysis of the data indicated a close association with peroxide values. This agrees with the findings of the few investigators *(29, 37)* who have examined the relationship. However, study of the data indicated that the high association may be somewhat superficial in nature. Total and monocarbonyl values often varied considerably at similar peroxide levels. The work of Nikkila and Linko *(38)* on vegetable oils indicated that peroxide-carbonyl relationships may differ in oils and fats of different origin and composition. The existence of differences in peroxide-carbonyl relationships would tend partly to explain the detection of rancidity at different peroxide levels *(36)*.

Data plotted in Figure 1 for a differently oxidized group of unrelated lard samples indicated variations in peroxide-carbonyl relationships. The greatest difference or variability was in the heated samples, and appeared to involve the monocarbonyl fraction. The dicarbonyl fraction showed a linear relation ship with peroxides. The variation possibly was due to differences in composition and stability of the lard samples.

Data shown in Figure 2 indicate peroxide-mono- and dicarbonyl relationships found for uncured and cured tissue samples from the same hog. (In this experiment, one bacon side was dry-cured with 4% salt and both sides were cut into six equal segments. The segments were stored at 15° F. and tested after 18 and 36 weeks of storage. Adjacent segments were distributed between the two storage periods. The first 3 points in each curve represent segments from 18 weeks and the last three from 36 weeks storage.) In a given group of samples, peroxide-monocarbonyl relationships tended to be linear in the early stages of oxidation. However, a difference in that relationship was indicated between uncured and cured samples. This was apparently

due to the pro-oxidant effect of the salt. There was little difference in the dicarbonyls of the two sets of samples, and relationships with peroxides were linear except in advanced stages of oxidation. Further work is required on the influence of composition, anti-oxidants, pro-oxidants, and other factors on peroxide-carbonyl associations. Such investigations seem necessary for a complete understanding of the chemistry of rancidity.

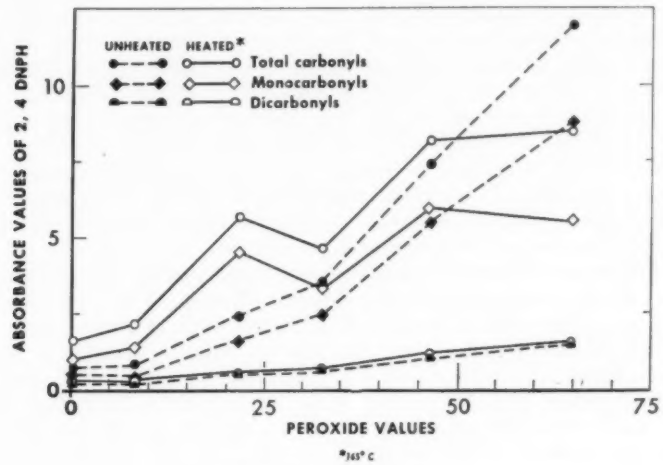


Figure 1. Oxidized lard.

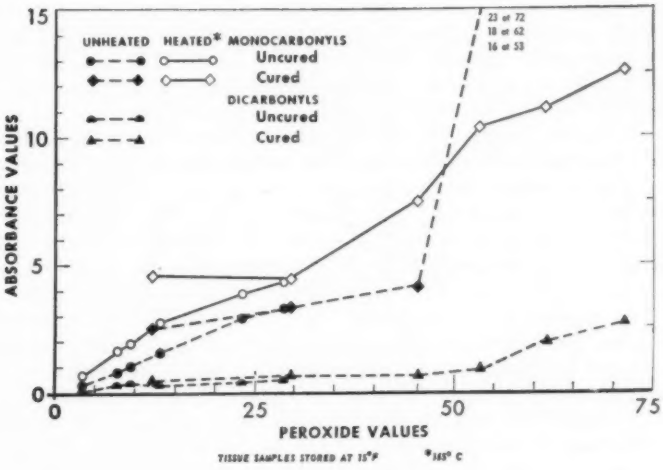


Figure 2. Paired bacon sides.

As indicated in an earlier report *(17)* total carbonyls of heated fat (Figure 1) increased at a more rapid rate than those of the unheated fat during the early stages of oxidation. This was also shown to be true in Figures 1 and 2 for monocarbonyls. However, in advanced oxidation, formation of carbonyls of unheated fat overtook and exceeded those of the heated fat. This curious behavior is probably partly due to volatilization and to decomposition, but it also appears to be chiefly a function of the degree of oxidation.

The monocarbonyl DNPH's gave the characteristic red color with alcoholic alkali. As reported earlier *(18)* the very small amount of monocarbonyls in the benzene extract so far have been found to consist entirely of low molecular weight saturated monocarbonyls. The dicarbonyl fractions gave blue colors in alcoholic alkali, which is characteristic of $\alpha$ dicarbonyls.

In general, the wavelength of maximum absorption of the unheated monocarbonyl fractions decreased with oxidation from 355 m$\mu$ to 343-6 m$\mu$, which indicated an increase in the proportion of saturated constituents. However, the wavelength of maximum absorption of the heated monocarbonyl fractions increased from 355 m$\mu$ to 365 m$\mu$ with oxidation. This shift indicated increases in proportions of unsaturated compounds.

**Separation and identification of monocarbonyl classes.** Paper chromatography of the monocarbonyls gave cleancut separations into 2 to 3 spots. Maxima were generally sharp and corresponded to those characteristic for saturated aldehyde, 2-en-1-al, and 2,4-dien-1-al classes. Braude and Jones *(5)* and others *(14, 15, 26)* have determined the characteristic range of maxima in ethanal and chloroform of the classes of 2,4-DNPH's. These values, along with determinations by the authors in carbon tetrachloride, ethanal, and alcoholic alkali, are shown in Table 1.

A spot was obtained from unheated fat and low oxidized heated fat that has not been completely identified. This fraction had maxima of 349-58 m$\mu$, which is intermediate between the saturated aldehydes and 2-en-1-als. This fraction was discussed by Gaddis and Ellis *(18)*, and the possibility considered that it might contain unconjugated unsaturated aldehydes. The $R_F$'s of this fraction were nearly identical with those of the 2-en-1-al class. It is now considered to be a mixture of methanol, ethanal, 2-en-1-als, and unknown compounds. The fraction will be further discussed below.

Table 2 shows a comparison of $R_F$ values obtained in the separation of mixtures of authentic DNPH's of as much as four classes of monocarbonyls, and the monocarbonyls from a sample of heated fat. This illustrates the effectiveness of the separation, with compounds separating into their proper classes with the exception of acetone, methanol, and ethanal *(19)*.

**Changes in the proportions of monocarbonyl classes.** Proportions of the different classes were determined by measurement of the absorbance values at the maxima, and calculation as per cent of the total amount recovered. Figures 3 and 4 show changes with oxidation in the major classes, in saturated aldehydes, and in 2,4-dienals of four different groups of samples. The proportion of saturated aldehydes (Figure 3) increased rapidly *(23, 28)*. However, in the heated fat, proportions of the saturated class generally decreased, but in late stages of oxidation showed a tendency to increase again slightly. Early in the oxidation, the 2,4-dienal class frequently was not detected, and heated monocarbonyls were similar in composition to those of the unheated.

**TABLE 1**

**Absorption maxima of classes of carbonyl 2,4-dinitrophenylhydrazones**

| | Authors | | | Braude and Jones | | Jones et al. (26) | | Forss et al. (14, 15) |
|---|---|---|---|---|---|---|---|---|
| | $CCl_4$ mμ | EtOH mμ | Alc. KOH mμ | $CHCl_3$ mμ | EtOH mμ | $CHCl_3$ mμ | Alc. KOH mμ | EtOH mμ |
| Aliphatic saturated aldehydes | 330-49 | 346-58 | 420-34 | 348-61 | 348-63 | 344-58 | 426-30 | 357-8 |
| Saturated methyl ketones | 349-52 | 358-61 | 434 | 365-8 | 362-5 | 364-7 | 430-34 | —— |
| 2-en-1-als [1] | 358-65 | 370 5 | 456-60 | 367-73 | 366-73 | 373 | 452-59 | 370-5 |
| 2,4-dien-1-als [1] | 380 | 389-90 | 480-4 | 388 | 379-85 | —— | | 389 |
| Mono α dicarbonyl | | | | 352 | 351 | | | |
| Bis α dicarbonyl | | | | 400 | | | | |
| Bis, unconjugated dicarbonyl | | | | 366 | | | | |
| Unknown dicarbonyls | 380-5 | | 545.50 | | | | | |
| Diacetyl | | 395* | | | | | | |

* Diacetyl in $CHCl_3$

[1] Furnished by E. L. Pippen, WURDD.

**TABLE 2**
**Comparison of separations into classes by paper chromatography at 4° C.**

| 2,4-DNPH's | $R_F$ values | | | | Max. |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | Av. | mμ |
| Mixture | | | | | |
| Saturated aldehydes, $C_3$-$C_{14}$ | 0.47 | 0.48 | 0.47 | 0.47 | See Table 1 |
| 2-en-l-als, $C_3$-$C_{12}$ | 0.37 | 0.36 | 0.35 | 0.36 | |
| 2,4-dien-l-als, $C_5$-$C_{12}$ | 0.21 | 0.23 | 0.23 | 0.22 | |
| Monocarbonyls | | | | | |
| From heated | 0.48 | 0.48 | 0.48 | 0.48 | 346 |
| Autoxidized stored (0° F.) | 0.35 | 0.37 | 0.36 | 0.36 | 361 |
| Tissue fat | 0.21 | 0.24 | 0.23 | 0.23 | 375-80 |
| Mixture | | | | | |
| Methyl ketones, $C_5$-$C_9$ | 0.58 | 0.56 | 0.57 | 0.57 | See Table 1 |
| 2-en-l-als, $C_3$-$C_{12}$ | 0.33 | 0.33 | 0.33 | 0.33 | |
| 2,4-dien-l-als, $C_5$-$C_{12}$ | 0.21 | 0.20 | 0.20 | 0.20 | |
| Mixture | | | | | |
| Methyl ketones, $C_5$-$C_9$ | 0.60 | 0.58 | 0.59 | 0.59 | See Table 1 |
| Saturated aldehydes, $C_1$-$C_{14}$ | 0.42 | 0.42 | 0.42 | 0.42 | |
| 2-en-l-als, $C_3$-$C_{12}$ | 0.32 | 0.30 | 0.31 | 0.31 | |
| 2,4-dien-l-als, $C_5$-$C_{12}$ | 0.17 | 0.17 | 0.17 | 0.17 | |

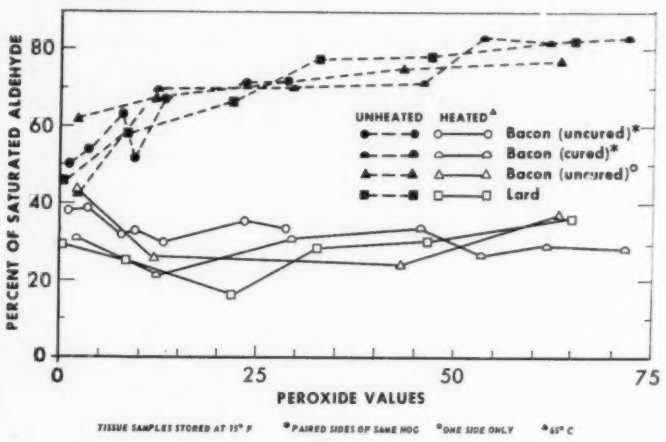


**Figure 3. Saturated aldehydes. Changes with oxidation.**

With oxidation the proportions of the 2,4-dienal class increased rapidly in the heated monocarbonyls. However, this class tended to decrease somewhat with advanced oxidation. There was considerable difference in the behavior of the different groups of samples. Interpretation of this requires further

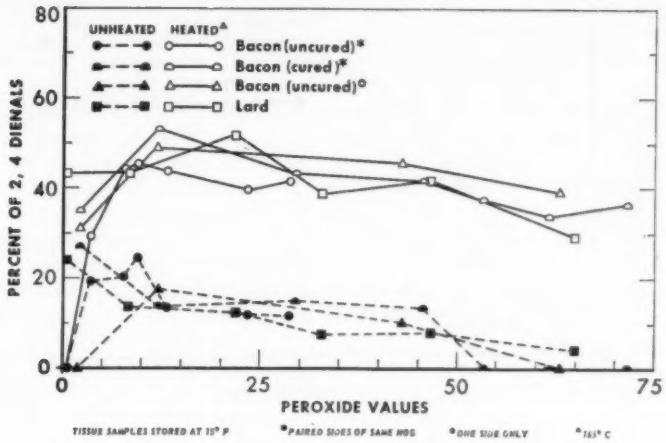


**Figure 4. 2,4 dienal. Changes with oxidation.**

study. Perhaps the greatest significance of these data is the rapid change in proportions that takes place during the initial stages of oxidation at the time rancidity first develops. Proportions of saturated aldehydes changed from 50% to 85% in the unheated, and 2,4-dienals from 0% to 55% in the heated fat.

**Oxidation and relative quantities of monocarbonyl classes.** The absolute absorbance values of the classes were determined from the total monocarbonyl absorbance values and the percentage proportions of the classes. Data shown in Figures 5, 6, and 7 are an extension of the experiments covered

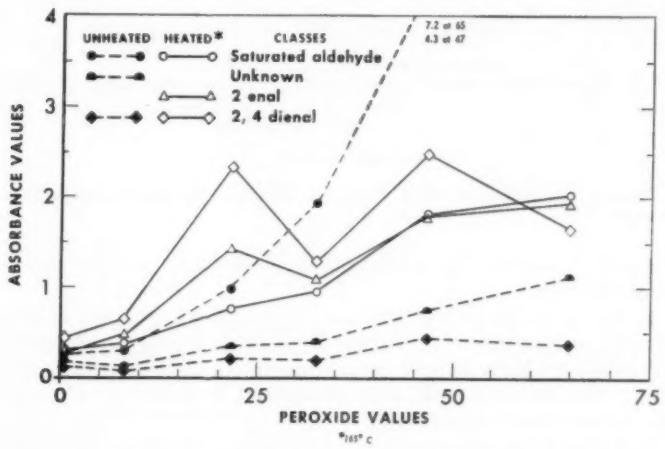


**Figure 5. Lard. Oxidation and relative amounts of monocarbonyls.**

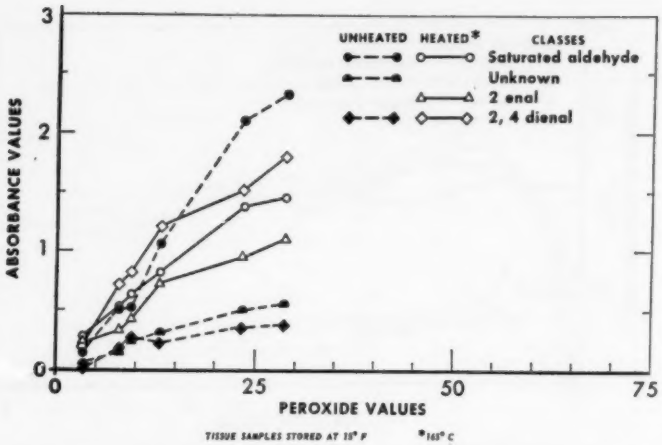


**Figure 6. Uncured pork fatty tissue. Oxidation and relative amounts of monocarbonyls.**

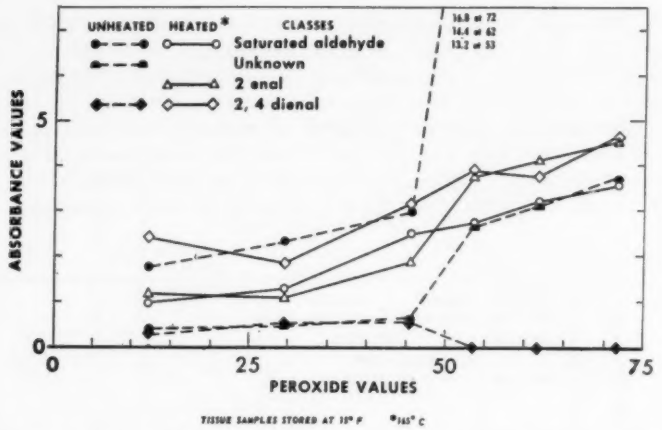


**Figure 7. Cured pork fatty tissue. Oxidation and relative amounts of monocarbonyls.**

by Figures 1 and 2. The absolute absorbance values are plotted against the peroxide values.

In Figure 5, the erratic behavior of the heated total and monocarbonyl discussed in connection with Figure 1 is indicated to be due primarily to the generation of 2,4-dienals in a manner unpredictable on the basis of the peroxides. The heated 2-enals also showed some variation. The rest of the class curves were fairly smooth.

In Figure 6, similar data for uncured bacon are given. The samples were from the same bacon side, and all monocarbonyl classes increased smoothly

with oxidation. These data illustrate particularly well the rapid increase and change in relative amounts of the monocarbonyl classes during the early stages of oxidation. Data shown in Figure 7 for cured bacon from the same animal (see Figure 7) are considerably different, although the fat was presumably similar in composition .Comparing Figures 6 and 7 over the same range of oxidation (peroxide 12-30), the rate of change and relative amounts of the classes are obviously different. It would appear that the pro-oxidant, salt, may influence the formation of oxidation products in some manner.

In over-all consideration of Figures 5, 6, and 7, there was a progressively increasing quantitative difference between the unheated and heated saturated classes. Although a large proportion of this class is composed of low molecular weight volatile compounds, volatilization during heating did not account for the decrease. Since the unheated saturated class increased tremendously in the late stages of oxidation, the disappearance on heating becomes very great, and probably accounts for the previously discussed reversal in quantitative relationship of the unheated and heated total monocarbonyls. Determination of the influence of fat composition and other variables on the relative amounts of the monocarbonyl classes would appear to be necessary for an understanding of the chemical nature of rancidity.

**Quantitative aspects of carbonyl determination.** A thorough study has been made of the reproducibility of the above determinations. This was found to usually not exceed ±3%. In the separation into classes, as shown by Gaddis and Ellis *(19)*, results for the 2,4-dienals will tend to be somewhat low and the other classes a little high. This is due to the particular sensitivity of the 2,4-dienals to light and air.

**Separation of classes into individual compounds.** Data shown in Figures 8 and 9 illustrate the separation by ascending paper chromatography *(13, 19)*

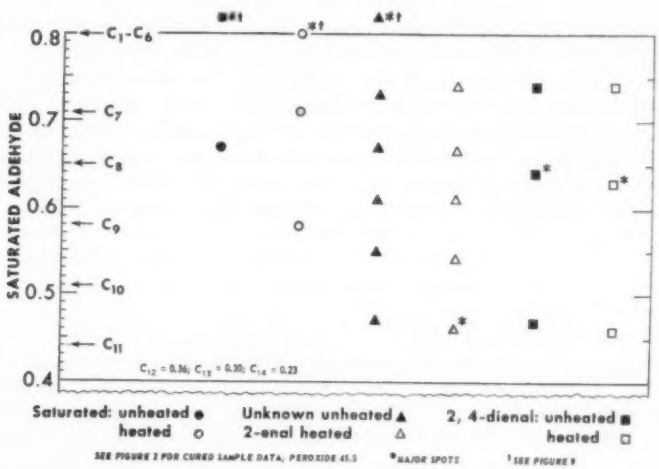


Figure 8. Vaseline-aqueous methanol system. Separation of classes into compounds, $R_f$ values.

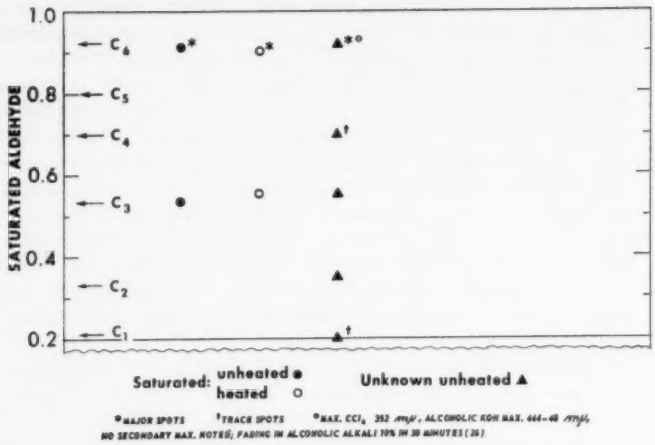


**Figure 9. Propylene glycol, Skellysolve C-Methanol. $R_f$ values of unseparated material from vaseline-methanol system.**

of the different classes of a sample of unheated and heated cured pork fat into compounds. On the left-hand side, the scale indicates $R_F$ values and the adjacent column a homologous series of authentic saturated aldehyde DNPH's. Unknown DNPH mixtures were applied first to the vaseline-aqueous methanol system (Figure 8), and after chromatography the unseparated material was transferred to the propylene glycol, Skellysolve C-methanol system (Figure 9). As indicated in Figure 8, higher molecular weight carbonyls were apparently similar in unheated and heated fats, with the exception of the saturated aldehydes. In that class, the unheated contained one compound and the heated two compounds. In the separation of lower molecular weight compounds shown in Figure 9, the heated contained none in the 2-enal class and was similar to the unheated in the saturated aldehyde class. The saturated aldehydes have been previously identified *(18)* as hexanal, propanal, ethanal, and methanal. Traces of actone and methyl ethyl ketone were also found. The two compounds with lowest $R_F$ values in the unknown class are unquestionably methanal and ethanal *(19)*. However, the ethanal might contain acrolein and the propanal traces of acetone. These results indicate the presence of 6 saturated aldehydes, two methyl ketones, three unknown unsaturated compounds, five 2-enals, and three 2,4-dienals; a total of at least 19 monocarbonyls in oxidized pork fat.

**Dicarbonyls.** The solubility of the dicarbonyl fractions (Figures 1 and 2) in common organic solvents indicated they are probably mostly composed of compounds of relatively long carbon chains. The fractions had maxima in carbon tetrachloride from 350-90 mμ. Maxima of unheated dicarbonyls were generally in the range of 380-90 mμ and showed a secondary peak at 430-40 mμ. Dicarbonyls from heated fat often showed a down shift to about 350-65mμ. According to Braude and Jones (5) (see Table 1) bis hydrazones

have normal absorption except those of α diketones which have maxima as high as poly-unsaturated monocarbonyls. Apparently the downward shift in maxima indicates the presence of small amounts of unconjugated dicarbonyls. Paper chromatography *(41)* gave three main fractions with maxima at 370, 385, and 390 mμ in carbon tetrachloride. These gave purplish-blue to blue colors in alcoholic alkali with maxima at 525, 545, and 550 mμ. Several minor fractions with maxima at 346 to 358 mμ preceded the α-dicarbonyls on the paper.

## SUMMARY

Methods are described for the estimation in fat of total steam volatile carbonyls, and mono- and dicarbonyl fractions thereof, as DNPH derivatives. The application of a new paper chromatographic method to the separation and determination of classes of monocarbonyl DNPH's is described. Classes of monocarbonyls changed progressively during oxidation with respect to those present and the proportions thereof. Heating oxidized fat at 165° C. also produced great changes in the classes of monocarbonyls. Types of monocarbonyls found were saturated aldehydes, minor amounts of the two lowest molecular weight saturated methyl ketones, a mixed group which contained as a major constituent a DNPH derivative of unknown class, 2-en-1-als, and 2,4-dien-1-als. In general, with oxidation, the proportion of saturated carbonyls increased in unheated fat, and unsaturated carbonyls (particularly 2,4-dienals) increased when the fat was heated. Resolution of the classes by paper chromatography indicated the probable presence in the saturated aldehyde class of not only hexanal, propanal, ethanal, and methanol, but at least two $C_7$-$C_9$ saturated aldehydes, and acetone, methyl ethyl ketone, three carbonyls of unknown class, five 2-en-1-als, and three 2,4-dien-1-als; a total of at least 19 monocarbonyls. The dicarbonyl fraction appeared to be composed principally of α-dicarbonyls, but there was evidence of the presence of other types of dicarbonyls.

The methods described are adapted to the determination of the kind and proportions of carbonyl classes formed in small amounts of fat as the result of conditions to which the fat or fatty tissues have been exposed. Application of these methods may shed light on the mechanism of rancidity development from the standpoint of peroxide-carbonyl relationships, the well-known sensitivity of taste and the classes of carbonyls present. The potential value of the procedures in the identification and determination of individual carbonyls is indicated.

## LITERATURE CITED

1. Anonymous. Experimental procedure for conducting taste and smell tests. U.S.D.A. Bureau of Human Nutrition and Home Economics 1943 (mimeo) 15 pp.
2. Bengtsson, K., and Helm, R. Principles of taste testing. *Wallerstein Lab. Commun.*, **9**, 171 (1946).
3. Bennett, A., May, L. G., and Gregory, R. A modification of the 2,4-dinitrophenylhydrazone method for determining total acetone and ketone bodies. *J. Lab. Clin. Med.*, **37**, 643 (1951).
4. Blakeslee, A. F. A dinner demonstration of threshold differences in taste and smell. *Science*, **81**, 504 (1955).
5. Braude, E. A., and Jones, E. R. H. Studies in light absorption Part II. 2:4 dinitrophenylhydrazones. *J. Chem. Soc.*, **382**, (84), 498 (1945).

6. BREKKE, J., AND MACKINNEY, G. Aldehydes from rancid corn and avocado oil. *J. Am. Oil Chemists' Soc.*, **27**, 238 (1950).
7. BUSS, C. D., AND MACKINNEY, G. Carbonyl compounds in a rancid corn oil. *J. Am. Oil Chemists' Soc.*, **32**, 487 (1955).
8. CHANG, S. S., AND KUMMEROW, F. A. The volatile decomposition products and the organoleptic characteristics of the oxidative polymers of ethyl linoleate. *J. Am. Oil Chemists' Soc.*, **30**, 251 (1953).
9. CHANG, S. S., AND KUMMEROW, F. A. The relationship between the oxidative polymers of soybean oil and reversion. *J. Am. Oil Chemists' Soc.*, **31**, 324 (1954).
10. CHANG, S. S., AND KUMMEROW, F. A. An instrumental method for measuring the degree of reversion and rancidity of edible oils. *J. Am. Oil Chemists' Soc.*, **32**, 341 (1955).
11. CHANG, S. S., AND KUMMEROW, F. A. A study of the flavor stability and autoxidation of beef fats. *J. Am. Oil Chemists' Soc.*, **32**, 547 (1955).
12. CHIPAULT, J. R. A study of the odor problems of fish oils. *Annual Report Hormel Inst.*, **61**, (1955-6).
13. ELLIS, REX, GADDIS, A. M., AND CURRIE, G. T. Paper chromatography of 2,4-dinitrophenylhydrazones of saturated aliphatic aldehydes. *Anal. Chem.*, **30**, 475 (1958).
14. FORSS, D. A., PONT, E. G., AND STARK, W. The volatile compounds associated with oxidized flavour in skim milk. *J. Dairy Research*, **22**, 91 (1955).
15. FORSS, D. A., PONT, E. G., AND STARK, W. Further observations on the volatile compounds associated with oxidized flavour in skim milk. *J. Dairy Research*, **22**, 345 (1955).
16. FORSS, D. A., DUNSTONE, E. A., AND STARK, W. Paper chromatography of alk 2:4 dienals. *Chem. & Ind. (London)*, **45**, 521 (1957).
17. GADDIS, A. M. Effect of pure salt on the oxidation of bacon in freezer storage. *Food Technol*, **6**, 294 (1952).
18. GADDIS, A. M., AND ELLIS, REX. Volatile saturated aliphatic aldehydes in rancid fat. *Science*, **126**, 745 (1957).
19. GADDIS, A. M., AND ELLIS, REX. Paper chromatography of 2,4-dinitrophenylhydrazones. The resolution of 2-alkanone, alkanal, alk-2-enal, and alk-2,4-dienal derivatives. *Anal. Chem.* **31**, 870 (1959).
20. GRIDGEMAN, N. T. Aspects of taste. *Wallerstein Lab. Commun.*, **20**, 203 (1957).
21. HANDSCHUMAKER, E. A technique for testing the reversion properties of hydrogenated soybean oil shortenings. *J. Am. Oil Chemists' Soc.*, **25**, 54 (1958).
22. HARRISON, J. B., AND DAUBERT, B. F. Flavor reversion in soybean oil VII. The autoxidation if isolinoleic acid. *J. Am. Oil Chemists' Soc.*, **30**, 371 (1953).
23. HENICK, A. S., BENCA, M. F., AND MITCHELL, J. H. Estimating carbonyl compounds in rancid fats and foods. *J. Am. Oil Chemists' Soc.*, **31**, 88 (1954).
24. HOPKINS, J. W. Precision of assessment of palatability of foodstuffs by laboratory panels. *Can. J. Research F24*, **203**, (1946).
25. JOHNSON, O. C., CHANG, S. S., AND KUMMEROW, F. A. Volatile decomposition products of the oxidation polymers of ethyl linolenate. *J. Am. Oil Chemists' Soc.*, **30**, 317 (1953).
26. JONES, L. A., HOLMES, J. C., AND SELIGMAN, R. B. Spectrophotometric studies of some 2,4 dinitrophenylhydrazones. *Anal. Chem.*, **28**, 191 (1956).
27. KAWAHARA, F. K., AND DUTTON, H. J. Volatile cleavage products of autoxidized soybean oil. *J. Am. Oil Chemists' Soc.*, **29**, 372 (1952).
28. KAWAHARA, F. K. DUTTON, H. J., AND COWAN, J. C. Volatile cleavage products of autoxidized methyl linolenate. *J. Am. Oil Chemists' Soc.*, **29**, 633 (1952).
29. KLOSE, A. A., HANSON, H. L., MECCHI, E. P., ANDERSON, J. H., STREETER, I. V., AND LINEWEAVER, H. Quality and stability of turkeys as a function of dietary fat. *Poultry Sci.*, **32**, 82 (1953).
30. LANG, D. A., AND PROCTOR, B. E. Some effects of ionizing radiations on lipids. I. Monocarbonyl production in vegetable oils. *J. Am. Oil Chemists' Soc.*, **33**, 237 (1956).
31. LEA, C. H. Recent developments in the study of oxidative deterioration of lipids. *Chem. & Ind. (London)*, **41**, 1303 (1953).
32. LIPS, H. J. Oxidative flavor deterioration in fats and oils. *Food in Canada*, **9**, (June 1952).

33. MORRIS, S. G. Fat rancidity. Recent studies on the mechanism of fat oxidation in relation to rancidity. *J. Agr. Food Chem.*, **2**, 126 (1954).
34. MOSER, H. A., JOEGER, C. M., COWAN, J. C., AND DUTTON, H. J. The flavor problem of soybean oil. II. Organoleptic evaluation. *J. Am. Oil Chemists' Soc.*, **24**, 291 (1947).
35. MRAK, E. M., AND STEWART, G. F. Editors. *Advances in Food Research*, Vol. 2. 1949. Academic Press, New York, N. Y.
36. NAUMANN, H. D., BRADY, D. E., PALMER, A. Z., AND TUCKER, L. N. Odor, flavor, and peroxide value as measures of rancidity in frozen ground pork. *Food Technol.*, **5**, 496 (1951).
37. NEUMER, J. F., AND DUGAN, L. R. A new method for measuring the development of rancidity. *Food Technol.*, **7**, 191 (1953).
38. NIKKILA, O. E., AND LINKO, R. R. Comparison of tests for oxidative rancidity in edible oils. *Suomen Kemistilehto*, **28B**, 113 (1955).
39. PIPPEN, E. L. NONAKA, M., JONES, F. F., AND STITT, F. Volatile compounds of cooked chicken 1. Compounds obtained by air entrainment. *Food Research*, **23**, 103 (1958).
40. POOL, M. F., AND KLOSE, A. A. Estimation of monocarbonyl compounds in rancid foods. *J. Am. Oil Chemists' Soc.*, **28**, 215 (1951).
41. RICE, R. G., KELLAR, G. J., AND KIRCHNER, J. G. Separation and identification of 2,4-dinitrophenylhydrazones of aldehydes and ketones, and 3,5-dinitrobenzoates of alcohols by filter paper chromatograph. *An. Chem.*, **23**, 194 (1951).
42. ROCKWOOD, B. N., RAMSBOTTOM, J. M., AND MEHLENSBACKER, V. C. Preparation of animal tissue fat for determination of peroxides and free fatty acids. *Ind. Eng. Chem., Anal. Ed.*, **19**, 853 (1947).
43. RUTMAN, J. Z. Method for the determination of acetone in two-tenths milliliter of blood. *J. Lab. Clin. Med.*, **41**, 648 (1953).
44. SCHEPARTZ, A. I., AND DAUBERT, B. F. Flavor reversion in soybean oil V. Isolation and identification of reversion compounds in hydrogenated soybean oil. *J. Am. Oil Chemists' Soc.*, **27**, 367 (1950).
45. STAPF, R. G., AND DAUBERT, B. F. Flavor reversion of soybean oil. VI. Isolation and identification of reversion compounds in unhardened soybean oil. *J. Am. Oil Chemists' Soc.*, **27**, 374 (1950).
46. SWIFT, C. E., O'CONNOR, R. T., BROWN, L. E., AND DOLLEAR, F. G. The aldehydes produced during the autoxidation of cottonseed oil. *J. Am. Oil Chemists' Soc.*, **26**, 297 (1949).
47. VAIL, G. E., AND CONRAD, R. M. Determination of palatability changes occurring in frozen poultry. *Food Research*, **13**, 347 (1948).
48. WITTIG, L. A. Chemical changes in fat. A. M. I. F. *Circ.* **35**, 77 (June 1957).

# THE FREE AMINO ACID PATTERN OF DATES IN RELATION TO THEIR DARKENING DURING MATURATION AND STORAGE[a]

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(Manuscript received July 14, 1958)

The darkening of dates involves at least two distinct processes: the formation of a dark layer under the skin resulting from enzymatic oxidation of the tannins that are localized in this zone, and the gradual darkening that takes place in the flesh as a whole towards maturity *(9)*. In contrast to the first process the latter cannot be attributed to a phenoloxidase system, since catechol tannins are absent in these tissues. On the other hand, favorable conditions for the Maillard reaction exist during ripening in the flesh of the fruit, i.e. the presence of reducing sugars and amino acids at a relatively high pH value and elevated temperature. Although a survey of the free amino acids in the ripe date *(Phoenix dactylifera)* has been reported recently *(4)* no information is available to date which might indicate their participation in the above processes. The present work, which reports the change of the free amino acid pattern during various phases of ripening, was undertaken as part of an investigation into the chemical constituents involved in the darkening of dates.

## EXPERIMENTAL

**Materials.** Dates (Deglet Noor variety) were obtained through the Date Administrative Committee, Indio, California. Samples were collected at intervals during maturation: when dates were uniformly green, yellow, red and brown, i.e., when harvested commercially. A commercial sample that had been stored for approximately 1 year at room temperature after the usual fumigation, cleaning and packaging processes and had darkened to a black-brown color was also investigated.

**Extraction.** Freshly pitted dates were macerated with 70% aqueous ethanol (2000 ml./kg. dates) and the mixture filtered. The filter cake was re-extracted with 1000 ml. aqueous ethanol and the combined filtrates were allowed to pass through a column of Dowex 50-8X resin in the H-form (radius: 1.4 cm.; height: 40 cm.). The column was washed successively with 1 liter of 70% aqueous ethanol and 2 liters of water. The basic components of the extract including all amino acids were then eluted with 1 liter of 1 *N* ammonia solution and the eluate concentrated at 30-40° under reduced pressure to a final volume representing 45 g. anhydrous dates per ml. This extract was used for paper chromatography and other experiments described below. The dry weight of the dates had been obtained by dehydration *in vacuo* over phosphorus pentoxide to constant weight.

## CHROMATOGRAPHY

Two-dimensional small-scale chromatography using Schleicher and Schuell 589 Blue Ribbon 12.5 cm. filter paper squares and the solvent systems; tert.-butanol (69.5 parts): water (20.5 parts): formic acid (1 part); and phenol (77.5 parts): water (21.5 parts): ammonia (1 part conc. solution) was carried out as described by Rockland and Under-

[a] Financial support for this work was provided by the Date Administrative Committee.

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wood *(11)*. Amino acids were identified by the position and color of their ninhydrin spots on the chromatograms relative to that of pure authentic compounds, by their behavior on ion exchange columns, by chemical degradation, and by special color reactions as described below. Typical chromatograms obtained by this method are shown in Figure 1. The amino acids found in such chromatograms are listed in Figure 2.

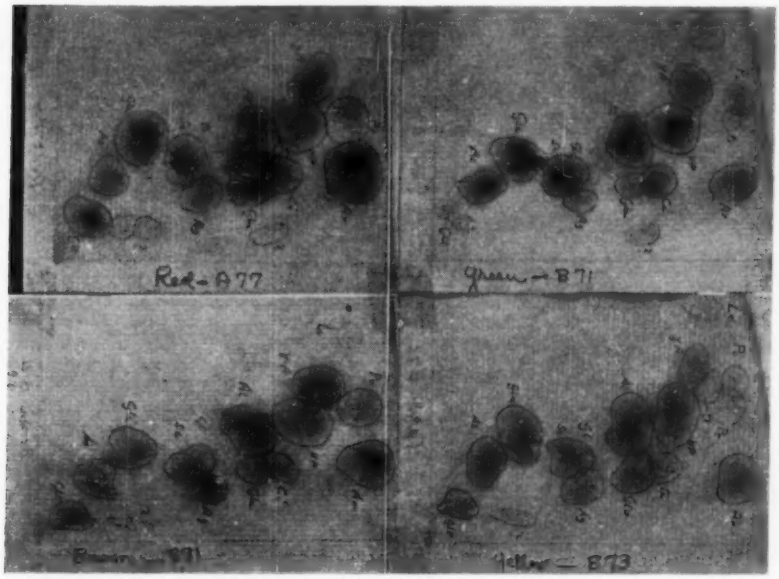


**Figure 1. Chromatograms of extracts from green, yellow, red and brown dates. Ag = asparagine, Al = alanine, Ar = arginine, As = aspartic acid, γ-A = γ-amino-butyric acid, B = baikiaine, Ci = citrulline, Gl = glycine, Gla = glutamic acid, Glt = glutathione (oxidized), Glu = glutamine, HP = 5-hydroxypipecolic acid, Le = leucine(s), Pi = pipecolic acid, Pr = proline, Se = serine.**

**Aspartic acid, glutamic acid, and glutathione (oxidized).** An IR-45 column (weakly basic resin) which had been washed with 1 *N* ammonia solution and water was charged with 1 ml. of the above concentrated extract and washed thoroughly with water. The effluent containing only neutral and basic amino acids was concentrated to 1 ml. and the concentrate chromatographed. The chromatogram clearly indicated the absence of the spots corresponding to oxidized glutathione, aspartic and glutamic acids. This was considered supporting evidence for the identity of the three acidic components present on the chromatograms of the original extract. Acid hydrolysis (heating for 2 hours at 100° C. with 6 *N* HCl) of date extract destroyed oxidized glutathione and led to the appearance of cystine on the chromatogram. Oxidized glutathione was characterized further by the iodoplatinate test *(15)* which was positive, and aspartic acid by its specific steel-blue color on development with ninhydrin and glutamic acid by its conversion to pyrrolidone carboxylic acid, i.e. its absence on the chromatogram after heating of the extract at pH 3 to 105° C. for 17 hours prior to application.

**Cystine.** This amino acid was identified by its $R_f$ values and its positive iodoplatinate test.

**Glutamine and asparagine.** One ml. of the above extract from which the acidic amino acids had been removed was now subjected to alkaline hydrolysis with 1 ml. 10 *N* NaOH at 100° C. for 1 hour. The reaction mixture was applied to a small Dowex 50-8X

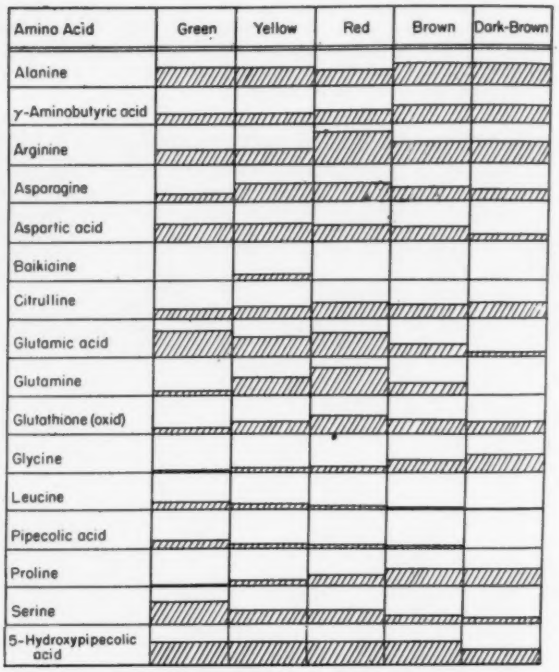


**Figure 2. Relative distribution of free amino acids in green, yellow, red, brown and dark brown dates determined by visual comparison of ninhydrin spot intensities of paper chromatograms.**

column and the column washed subsequently with water. The amino acids were then eluted from the resin with 1 *N* ammonia solution while Na ions were retained by the resin. The eluate was concentrated to 1 ml. and chromatographed. Aspartic and glutamic acids, the hydrolytic products of asparagine and glutamine, reappeared on the chromatogram thus confirming the presence of the latter two amino acids in the original extract. The presence of asparagine in date extracts was further supported by its characteristic orange-brown ninhydrin color which turns violet on standing and blue on heating.

**Arginine.** Following the above alkaline hydrolysis of date extract a new spot corresponding to ornithine was observed, evidently resulting from the degradation of arginine which was now missing from the chromatogram. When date extract was passed through a column of IRC-50 resin (weakly acidic) which had been buffered at pH 4.8 with sodium acetate buffer, the only amino acid missing from the chromatogram was the one tentatively identified as arginine. This observation established the basic character of the substance represented by this spot. Its identity with arginine was further substantiated by a positive color test with 8-hydroxyquinoline according to Jepson *(7)*.

**Citrulline.** When a date chromatogram was dipped in a solution of Ehrlich's reagent (1 g. dimethylaminobenzaldehyde, 2 ml. conc. HCl in 100 ml. ethanol) a bright yellow spot appeared immediately upon drying, possessing $R_f$ values corresponding to those of citrulline. It was partially superimposed on the glutamine spot, which appeared after development with ninhydrin reagent. Authentic glutamine, histidine, or ornithine did not give a yellow spot with Ehrlich's reagent. Since this amino acid had not been reported hitherto in fruit other than the watermelon, it appeared important to exclude the possibility of an

artifact. That citrulline could not have arisen from arginine during elution from the Dowex 50-8X column with ammonia (cf. Fox, 2) was shown by passage of authentic arginine through this resin under identical conditions which produced, however, no trace of citrulline. Acid hydrolysis (date extract + 1 volume of conc. HCl heated at 100° C. for 18 hours) converted citrulline quantitatively to ornithine, while arginine was not affected by this treatment. Alkaline hydrolysis under the conditions described above, on the other hand, led to complete degradation of arginine to ornithine while citrulline remained apparently unaffected. Authentic citrulline likewise withstood destruction under these conditions.

**5-Hydroxypipecolic acid.** This amino acid, which was first discovered by Gartler and Dobzhansky *(3)* and later isolated by Virtanen and Kari *(17)* and independently by Grobbelaar *(5)* and Grobbelaar *et al. (4)*, was obtained from dates by a procedure recently described by Witkop and Foltz *(19)*. Its hydrochloride had mp: 218-222° C. with decomposition (uncorrected). On sublimation *in vacuo* it lost hydrochloric acid and gave the free amino acid. The $R_f$ values for 5-hydroxypipecolic acid in tert.-butanol: water: formic acid/phenol: water: ammonia are 0.49/0.68. It gives a red violet characteristic stain with ninhydrin similar to pipecolic acid, a sky blue spot with isatin and a deep burgundy-colored stain after successive treatments of the chromatogram with Ehrlich's reagent and ninhydrin. This stain remains without fading for many months.

**γ-Aminobutyric acid.** This amino acid was identified by its $R_f$ values and by co-chromatography with an authentic sample of this compound. It was observed that this amino acid, in contrast to valine from which it is difficult to separate with the solvent systems used in this work, gives a light yellow spot after development on the chromatogram with Ehrlich's reagent. This stain develops gradually over a period of 24 hours or longer and does not represent a very sensitive test for γ-aminobutyric acid.

**Proline.** Was recognized by its $R_f$ values, its characteristic yellow color with ninhydrin and dark blue stain with isatin reagent.

**Serine and glycine.** The identification of these two amino acids which are difficult to separate from one another was based mainly on the relative positions of the ninhydrin spots on the chromatograms. Pronounced differences in intensity of the two spots fortunately facilitated their distinction considerably. Glycine was also recognized by its green coloration with the phthalic dialdehyde-urea reagent *(1)*.

**Alanine.** The identity of this amino acid was ascertained solely by its $R_f$ values and by co-chromatography with authentic alanine.

**Baikiaine.** The presence of trace amounts of this very rare amino acid in yellow dates was indicated by an orange-yellow spot *(5)* with the approximate $R_f$ values of 0.55/0.84 in the above solvent systems. It could not be detected in extracts from dates at other stages of maturity.

## DISCUSSION

As this investigation was primarily concerned with the possibility of amino acid participation in browning reactions, our interest was focussed mainly on gross changes of the amino acid composition during normal and excessive pigment formation in the fruit. Accordingly, variations in the relative amounts of each constituent during the indicated ripening phases were roughly estimated by visual comparison of their spot intensity on two-dimensional chromatograms (Fig. 1.). Such variations are represented by the shaded areas in Fig. 2. Examination of this table reveals definite trends in the amino acid pattern during maturation. Thus, aspartic and glutamic acid, serine, leucine and pipecolic acid decrease while γ-aminobutyric acid, glycine and proline increase during the ripening process (green-yellow-red-brown). Alanine, citrulline and 5-hydroxypipecolic acid show little change. The most striking feature in this panorama of free amino acids in the ripening fruit is, however, the sudden and sharp rise of glutamine, arginine, and glutathione during the red phase and their decline in the brown stage of maturing. This trend is continued in the mature fruit during deterioration on storage to a black-brown

date of inferior flavor and quality. Glutamine is absent entirely at this stage, glutathione has decreased to a low level and asparagine as well as most other amino acids appear to have diminished somewhat in concentration. The most evident change during this phase is, undoubtedly, the drastic reduction of aspartic and glutamic acid which gave prominent spots — although of steadily decreasing intensity — throughout the maturing period. The significance of these observations in relation to browning and excessive darkening will, of course, not become apparent until a study of other constituents (tannins, organic acids etc.) involved in these processes has been completed. However, the reported decrease in pH value *(12)* which parallels the progressive darkening of ripe dates on storage may well be relevant to the above findings.

It is generally held that the most important coloring matter in dates arises from the tannins located in the epiderm and first layer of the hypoderm. These compounds are oxidized by a polyphenolase during ripening as pointed out above and yield insoluble dark products. However, continued darkening after thermal destruction of the enzyme can be observed and it is thus likely that other as yet unknown reactions may be involved in the formation of these dark pigments. Indirect evidence mentioned in a report on structure and chemical factors in relation to fungus spoilage of dates *(16)* and some preliminary chromatographic results obtained with date extracts by Jurd of this laboratory indicate the presence of phloroglucinol or similar phenols in the ripe date. The possibility of a Maillard-type interaction between such phloroglucinol derivatives and amino acids to give dark colored products has been pointed out recently by Rinderknecht and Jurd *(10)* who demonstrated the formation of dark products in several buffer solutions (pH 6-8) of phloroglucinol and glycine on standing at room temperature. Although experimental evidence for the occurrence of this reaction in ripening fruit is as yet lacking, the presence of phloroglucinol derivatives in fruit and the results of the above model experiments provide a rational basis for the investigation of such an interaction in plant material.

On comparison of the free amino acid chromatograms obtained in the present study with that reported by Grobbelaar *et al. (4)* several notable differences become apparent. Weak spots representing threonine, lysine, valine, $\beta$-alanine and homoserine are indicated on the chromatogram of these authors, but no supporting evidence for their identity is given. We have been unable to confirm the presence of these amino acids in our date extracts. We have, on the other hand, reasonable chromatographic and chemical evidence for the occurrence in our date extracts of glutathione, glutamine and citrulline. These amino acids, although present in fairly high concentration, are not listed by Grobbelaar *et al.* We also found high levels of arginine in all our extracts in contrast to the trace amount indicated on the chromatogram of the above authors. This, however, may be due to the use of Zeorex resin by the latter for purification of their extracts. Zeorex, a phenol-sulfonic acid resin is known to bind a large part of the basic amino acids irreversibly. Some of the differences mentioned above may also be due to the use of a different variety of date in the study by Grobbelaar *et al.*

In conclusion, special mention should be made of two amino acids occurring in dates which are of particular interest although not connected with

the object of this work, i.e. citrulline and 5-hydroxypipecolic acid. The latter which occurs in relatively high and practically constant concentration in dates throughout the maturing period originates probably from δ-hydroxylysine *(18)* and raises numerous questions as to its metabolic role in plants. In view of the growth-inhibiting activity reported for its lower homologue, hydroxyproline *(14)*, a study of the potential anti-metabolite effect of 5-hydroxypipecolic acid might be rewarding. Citrulline which, up to the present has not been found in fruit other than the watermelon is an intermediate in the well known Krebs-Henseleit cycle of urea synthesis. According to Isawa *(6)*, citrulline when given subcutaneously increases distinctly urine output in normal persons and animals. Its diuretic action is apparently mediated through increased urea formation by the liver. It is tempting in this connection to explain the therapeutic effect of watermelon juice in nephrosis in terms of its citrulline content. The role of citrulline in the biosynthesis of pyrimidines in the pigeon and rat has been investigated and reviewed recently by Smith and Stetten *(13)*. Citrulline has also been found to possess cancer-inhibiting activity *(8)*. Like nitrogen mustard N-oxide, azaguanine, 2, 4-bis-ethylene-imino-hydroquinone etc., citrulline caused depolymerization and degradation of deoxyri-bonucleic acid in cancer cells and disappearance of ribonucleic acid. It may be anticipated that further studies of the biochemical and physiological role of this important amino acid will yield interesting results. Elucidation of its function in the ripening date fruit might be one such contribution.

## LITERATURE CITED


1. Curzon, G., and Giltrow, J. Aromatic aldehydes as specific chromatographic color reagents for amino acids. *Nature*, **173**, 314 (1954).
2. Fox, S. W. The preparation of citrulline by hydrolysis of arginine. *J. Biol. Chem.* **123**, 687 (1938).
3. Gartler, S. and Dobzhansky, T. Excretion in human urine of an unknown amino acid derived from dates. *Nature*, **174**, 553 (1954).
4. Grobbelaar, N., Pollard, J. K., and Steward, F. C. New soluble nitrogen compounds in plants. *Nature*, **175**, 703 (1955).
5. Grobbelaar, N. (Ph.D. thesis, Cornell University, Ithaca, N.Y., 1955).
6. Isawa, T. The diuretic action of watermelon. Japan *J. Med. Sci.* VIII, Internal Med. Ped. Psychiatr., 5, *Proc.*, **90**, 101 (1938).
7. Jepson, J. B. A fluorescence test for serotonin and other tryptamines. Multiple dipping procedures in paper chromatography: A specific test for hydroxyproline. *Nature*, **172**, 772 and 1100 (1953).
8. Kotobuki, H. and Mimura, F. Metabolism of cancer tissues. II. The effect of cancer-inhibiting substances and amino acids on ascites hepatoma. *Folia Pharmacal., Japan*, **51**, 149 (1955).
9. Rashid, I. A. (Ph.D. thesis, University Massachusetts, Amherst, Mass. 1950).
10. Rinderknecht, H. and Jurd, L. A novel non-enzymic browning reaction. *Nature* **181**, 1268 (1958).
11. Rockland, L. B., and Underwood, J. C. Small-scale filter paper chromatography: A rapid 2-dimensional procedure. *Anal. Chem.* **26**, 1557 (1954).
12. Rygg, G. L. Date Growers Institute, Indio, Calif. *33rd Ann. Report*, p. 8 (1956).
13. Smith, L. H., and Stetten, D. Biosynthesis of orotic acid from citrulline. *J. Am. Chem. Soc.*, **76**, 3864 (1954).
14. Steward, F. C. (unpublished results).
15. Toennies, G. and Kolb, J. J. Techniques and reagents for paper chromatography. *Anal. Chem.*, **23**, 823 (1951).

16. TURRELL, F. M., SINCLAIR, W. B. AND BLISS, D. E. Date Growers Institute, Indio, Calif. 17. *Ann. Report*, p. 9 (1940).

17. VIRTANEN, A., AND KARI, S. 5-Hydroxy-piperidine-2-carboxylic acid in green plants. *Acta. Chim. Scand.*, **8**, 1290 (1954).

18. VIRTANEN, A., AND KARI, S. 4-Hydroxy-piperidine-2-carboxylic acid in green plants. *Acta. Chim. Scand.*, **9**, 170 (1955).

19. WITKOP, B., AND FOLTZ, C. M. The configuration of 5-hydroxypipecolic acid from dates. *J. Am. Chem. Soc.*, **79**, 192 (1957).

# FLAME PHOTOMETRIC DETERMINATION OF CALCIUM, MAGNESIUM, AND POTASSIUM IN CANNED TOMATOES

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(Manuscript received August 7, 1958)

Riper tomatoes are soft in texture and tend to break up during canning. Kertesz *et al. (9)* reported that treating peeled tomatoes with small quantities of calcium salts improved the firmness of the canned product. This improvement was attributed to the reaction of the added calcium with the pectic acid present in the fruit to form a gel which supported the cell structure against the destructive action of heat. Thus, precise control of the calcium content of canned tomatoes is important to the quality and grade of the product.

The official method for the quantitative analysis of calcium includes precipitating the calcium as oxalate from the ashed sample, and then titrating the oxalate with potassium permanganate or weighing the ignited product as calcium oxide *(1)*. For determining certain metallic ions in foods, analysis by the flame photometric method has been shown to be more accurate than the colorimetric method *(10)*. Reports on flame photometric determination of calcium *(2, 3, 4, 5, 6)*, sodium, and potassium *(3, 4, 5, 7, 8, 10)* have been published. However, little is known of the applicability of this method to canned whole tomatoes.

In the investigation reported herein, the flame photometric method was used to determine calcium, magnesium, and potassium in canned whole tomatoes. In addition, the versene extraction method of determining calcium is compared with the ashing method.

## MATERIALS AND METHODS

**Canned tomatoes.** Commercial samples of whole tomatoes, packed in No. 303 cans, were purchased from the market. The tomato-juice sample was produced during a previous investigation.

**Ashing.** The entire contents of each can were disintegrated in a Waring blender. Duplicate 10-g. portions of the samples were weighed accurately in silica crucibles and evaporated to dryness on a steam bath. The residue was heated slowly on a hot plate until a slight charring occurred; it was then ashed in an electric muffle furnace at 525° C. until a constant weight was obtained. The ash was weighed and then dissolved in 0.2 N HCl for subsequent determinations of calcium, magnesium, and potassium by flame photometry.

**Flame photometry.** A Beckman DU spectrophotometer, adapted for flame analysis, was used *(4)* with the following wavelengths: Ca, 422.7 mμ, low sensitivity; Mg, 285 mμ, high sensitivity; and K, 767 mμ, high sensitivity with a blue filter. The pressures were adjusted to 10 p.s.i.g. for oxygen and 5 p.s.i.g. for acetylene. Standard curves were made with concentrations of Ca = 0, 50, 100, 200, and 300 p.p.m.; Mg = 0, 20, 50, and 100 p.p.m.; and K = 0, 300, 500, and 800 p.p.m.

A phosphorus-sulfur-nitrogen (PSN) reagent was used for a better resolution of the peaks of the emission curve. This reagent contained 50 p.p.m. of P ($H_3PO_4$), 20 p.p.m. of S ($H_2SO_4$) and 10 p.p.m. of N ($HNO_3$).

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If the concentrations of the unknown solutions were out of range, a diluent made from 700 ml. of 1.5 N HCl, 175 ml. of PSN solution, and 2,625 ml. of distilled water, was used. Twenty ml. of the sample solution (containing the salts) was pipetted into a test tube and mixed with 1 ml. of the PSN solution. The mixture was used directly for the flame photometry, and the results were expressed as milligrams of the element per 100 g. of the tomato product.

**Standard curve for calcium in a 1% versene solution.** In the direct extraction method of calcium determination, the high sodium content derived from the tetra sodium salt of ethylenediaminetetra acetic acid (EDTA or versene) caused severe emission interference. A standard curve, made from a solution containing 0, 50, 100, 200, and 300 p.p.m. of calcium in 1% versene, was constructed. Ten ml. of the synthetic juice, containing 3.0% sucrose, 0.238% potassium, 0.010% magnesium and 1.0% versene, was used to make 100 ml. of the standard solution.

**Flame photometry of calcium in versene solution.** Ten grams of the tomato product was diluted to 100 ml. with a 1% versene solution in a volumetric flask. The mixture was centrifuged and filtered through a Whatman No. 1 filter paper. Twenty milliliters of the filtrate was mixed with 1 ml. of PSN solution and used for the flame photometry of calcium at 422.7 mμ. The standard curve for calcium in the synthetic juice was used to compensate for the interference due to sodium, versene, magnesium, potassium, and sugar.

## RESULTS AND DISCUSSION

**Calcium, magnesium, and potassium in canned tomatoes.** Flame photometric analyses of the calcium, magnesium, and potassium content of the canned tomatoes and juices are shown in Table 1. The ash content

**TABLE 1**

**Flame photometric determination of calcium, magnesium, and potassium in canned tomatoes**

| Sample | Product | Soluble solids, Brix at 20° C. | Ash, % | Calcium, mg./100 g. (422.7 mμ) | Magnesium, mg./100 g. (285 mμ) | Potassium, mg./100 g. (767 mμ) |
|---|---|---|---|---|---|---|
| A | Canned whole tomatoes | 6.5 | 0.750 | 44.3 | 9.9 | 238.4 |
| B | Canned whole tomatoes | 6.5 | 0.798 | 39.3 | 11.8 | 238.8 |
| C | Canned tomato juice | 7.5 | 1.293 | 4.0 | 9.9 | 281.2 |

of the whole tomatoes was lower than that of the juice. The difference was presumably caused by the sodium chloride, which was added to the juice only during the processing. The calcium content of the peeled whole tomatoes was about ten times higher than that of the juice, which indicated that calcium salt was added during canning. The magnesium content of the samples differed slightly. Potassium was found to be present in larger amounts than any other element.

**Extraction of calcium from canned tomatoes with versene.** In order to test the applicability of the versene extraction method for flame photometric determination of calcium in tomatoes without the ashing procedure, the

same samples were analyzed for calcium content with and without ashing. It was discovered that the use of a 1% versene solution produced an error in the flame intensity of calcium. This was presumably caused by the presence of a large amount of sodium, which was introduced as the tetra sodium salt of EDTA during extraction. To compensate for the ionic interference and viscosity effect of versene on the emission intensity of calcium, a standard curve was constructed with calcium contents varying from 0 to 300 p.p.m. in 1% versene and 10% by volume of a synthetic juice as described previously. The result is shown in Fig. 1. The relationship between emission intensity

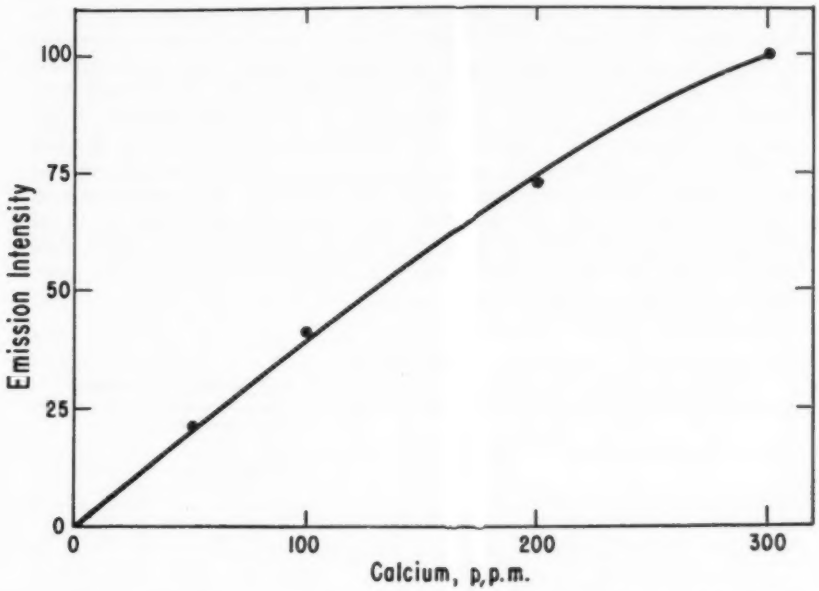


**Figure 1. Emission curve of calcium in 1% versene solution containing 10% of a synthetic tomato juice.**

and calcium concentration was not a linear function. This was probably due to self-interference, or to non-linearity of the light emission at different concentrations of calcium as a result of self-absorption and altered dissociation. Radiation interference due to ionized particles could also have contributed to the non-linearity of the curve. Chow and Thompson *(6)* found similar results in the emission intensity of calcium at different concentration levels, and attributed these to a positive radiation interference with calcium emission by sodium.

**Flame photometric determination of calcium in tomatoes.** Recently, Bauserman and Olson *(2)* reported on the flame photometric determination of calcium in sugar beet pulp by the versene extraction procedure instead of by the ashing method. In order to apply their method to the determination

of calcium in canned tomatoes, experiments were designed to test the accuracy of the versene method as compared with the ashing procedure. The results are shown in Table 2. It is apparent that versene extraction gave poorer results than ashing. Similar results were obtained by Bauserman and Olson *(2)* on the calcium content of wheat and milo. The calcium contents of these grains, as determined by the ashing procedure, were 0.052 and 0.036%, while those obtained by the versene method were 0.048 and 0.032%. It is not clear why the versene extraction method gave lower results, since the same sample and the same flame photometer were used in the analysis. The difference may have been due to: 1) incomplete extraction of calcium from the tomato pulp by versene; 2) radiation interference on the calcium emission due to the large amount of sodium or EDTA, or both; or 3) some unknown constituents in tomatoes which interfered with the calcium emission and were removed only by ashing. The accuracy of the flame photometric determination of cations in biological fluids is also dependent on 1) constant delivery of the sample to the burner; 2) correction for interference in the flame; 3) accurate isolation of the emissions; 4) atomization pressure; 5) solution viscosity; 6) nozzle radius; and 7) temperature. Among these, the interferences due to other elements or molecular bands are more important than others. When many analyses of a few similar materials are to be made, the versene extraction method might be used for routine analysis provided the error due to interference is properly compensated for.

**TABLE 2**

**Flame photometric determination of calcium in canned whole tomatoes**

| | | Versene Extraction method (standard calcium solution with no versene) | | Versene Extraction method (standard calcium solution in 1% versene and 10% synthetic tomato juice) | | Ashing method |
|---|---|---|---|---|---|---|
| Sample | Ash % | E, % [1] | Calcium, mg./100 g. | E, % [1] | Calcium, mg./100 g. | Calcium, mg./100 g. |
| A | 0.750 | 44.0 | 112 | 13.0 | 32.0 | 44.3 |
| | | 44.0 | 112 | 13.0 | 32.0 | 44.3 |
| B | 0.798 | 46.0 | 119 | 12.0 | 28.0 | 39.3 |
| | | 46.0 | 119 | 12.0 | 28.0 | 39.3 |

[1] Emission intensity for calcium at 422.7 mμ

Although the addition of calcium salts to peeled whole tomatoes could increase the number of intact tomatoes, and improve the texture of the canned product, an excessive amount could cause a bitter flavor and an undesirable leathery texture *(9)*. For quality control purposes, proper control of the amount of calcium in the canned product is necessary.

## SUMMARY

The flame photometric method was used to determine the calcium, magnesium, and potassium contents of canned whole tomatoes. The samples used in this investigation contained 39.3 to 44.3 mg. of calcium, 9.9 to 11.8 mg. of magnesium, and 238.8 mg. of potassium per 100 g. of sample. The calcium

content of commercial canned whole tomatoes was about ten times higher than that of tomato juice, which indicates that calcium salt was added during canning. The versene extraction method and the ashing method were compared for flame photometric determination of calcium in canned tomatoes. The versene method gave lower results than ashing, presumably because of an interference in the calcium spectrum caused by the large amount of sodium, introduced as the tetra sodium salt of EDTA, and other constituents present in tomatoes. Factors influencing the emission intensity of calcium are discussed. The importance of accurate control of the calcium content in canned tomatoes is also indicated.

**Acknowledgment**

The authors wish to thank Mr. James W. Perdue of the Department of Vegetable Crops for his kind interest and help in this work.

## LITERATURE CITED

1. Assoc. Offic. Agr. Chemists, *Methods of Analysis*, 8th ed., 104 (1955).
2. Bauserman, H. M., and Olson, R. F. Analyses of Plant Materials Using EDTA Salts as Solubilizers, *J. Agr. Food Chem.*, **3**, 942 (1955).
3. Bernstein, R. E. Correction of sources of error in the estimation of sodium, potassium, and calcium in biological fluids and tissues by flame spectrophotometry. *Biochim. Biophys. Acta*, **9**, 576 (1952).
4. Brown, J. G., Lileland, O., and Jackson, R. K. Use of flame methods for analysis of plant material for potassium, calcium, magnesium, and sodium. *Proc. Amer. Soc. Hort. Sci.*, **56**, 12 (1950).
5. Burriel-Marti, F., and Ramirez-Munoz, J. *Flame Photometry, a Manual of Methods and Applications*. 1957. Elsevier Publishing Co. New York, N. Y.
6. Chow, T. J., and Thompson, T. G. Flame photometric determination of calcium in sea water and marine organisms. *Anal. Chem.*, **27**, 910 (1955).
7. Fox, C. L. Stable internal standard flame photometer for potassium and sodium analysis. *Anal. Chem.* **23**, 137 (1951).
8. Gehrke, C. W., Affsprung, H. E., and Wood, E. L. Flame photometric determination of potassium with ion-exchange separation of interfering anions. *J. Agr. Food Chem.* **3**, 48 (1955).
9. Kertesz, Z. I., Tolman, T. G., Loconti, J. D., and Ruyle, E. H. *The use of calcium in the commercial canning of whole tomatoes.* New York State Agr. Expt. Sta., Tech. Bull. No. 252 (1940).
10. Wills, P. A. Sodium and potassium in Australian foods. *Australian J. Exptl. Biol. Med. Sci.*, **34**, 165 (1956).

# GRAPHIC MEASUREMENTS OF UNMOLDED GELS[a]

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(Manuscript received March 31, 1958)

Physical qualities of agar, pectin and gelatin gels are important because of the direct influence on general acceptance. Many methods have been developed to measure firmness of gels and the literature is extensive on this subject. The elastic properties of gels permit several types of measurements. These are essentially stress-deformation methods, for example, use of a plunger against the surface of the jelly *(5)*, rupture of a slice of gel or the surface of the gel *(1, 2)*, resistance to movement of vanes placed in the gel when torque is applied *(4, 7)*, and change in height of a gel when unmolded using the instrument commonly called the "Ridgelimeter" *(3, 6)*.

Food manufacturers are not interested in empirical tests of the kind mentioned as they are in "use" tests directly related to those properties important to the consumer. The measurements can be completely arbitrary provided they measure those qualities that are important. There are many examples of so-called empirical methods which have been modified to give a "use test". A notable example is the method which requires a simple measurement of change in height of an unmolded gel for a firmness determination *(3)*.

A popular method of serving gels is in the unmolded form. The consumer requires a firm gel that is easily unmolded and shows a minimum amount of relaxation or sag under its own weight. Non-melting type gels (agar, alginate, pectin) show pronounced initial sag but little or no further decrease even at room temperature. In such gels the total change is due to relaxation of the elastic forces. Melting type gels (gelatin) show initial sag and in addition when above a critical temperature there is further decrease in height due to melting. The measurement of the change in height by a micrometer measurement gives an incomplete characterization of the gel. The change in the base of the gel, the tilting and other changes in the shape of the gel are important and absent in such simple measurements. To distinguish between sag due to relaxation of the elastic forces and sag due to melting effects, the terms "initial sag" and "thermal sag" are used in the Jell-O Division Research Laboratories.

Photographs of the unmolded gels taken at periodic intervals were used in the past to record the changes taking place. This proved to be a practical method but it required more or less subjective measurements to identify small improvements or deficiencies. It was also difficult to systematically follow the effects of formula variations such as pH and concentration.

This laboratory investigated several alternative methods for the objective measurement of sag and change of shape of unmolded gels. The Ridgelimeter

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[a] Presented at the 18th Annual Meeting of IFT, Chicago, May 27, 1958.

[b] Present address: American Machine & Foundry Co., Commercial Development Division, Springdale, Conn.

was found to be satisfactory only for relatively firm gels which show mostly initial sag and little or no thermal sag. This limited its use in experimental work where a wide range of gel properties are encountered.

Some modifications of the Ridgelimeter were evaluated such as (a) micrometer depth reading and change in base, (b) casting of shadow lines of the mold on a frosted glass plate and direct tracing of the lines or (c) photograph of the gel in front of a scale marker to show height. None of these modifications were adequate for our purposes. Accordingly, this laboratory developed a special test cabinet designed to:

(1) be capable of obtaining a complete outline of the unmolded gel,
(2) have automatic features to correct for tilting and distortion of the unmolded gel,
(3) have flexibility so that several different samples can be run at the same time,
(4) make measurements under fixed conditions of temperature and relative humidity, and
(5) have facilities to change conditions readily.

## Equipment and Methods

**General.** The special test cabinet consists of a series of 4 temperature-and humidity-controlled test compartments each of which have provisions for casting a succession of shadows on the same photographic plate without disturbing the sample or opening the compartment. These measurements can be made over a wide range of temperatures and relative humidities. A front panel view of the test cabinet showing general compartment detail is given in Figure 1.

The test cabinet can be broken down into two essentially independent parts: the atmosphere control section and the test compartment section. The atmosphere control

**Figure 1. Test cabinet.**

section consists of the electronics, the duct work and the other components necessary to adjust and control the atmospheric conditions desired in the test compartments. The test compartment section consists of the mechanical, optical and photographic accessories which make possible the recording of the sag and change of shape of the unmolded gels under the specified conditions. A detailed description of each portion follows.

**Atmosphere control section.** The physical layout and the operation of the atmosphere control section of the test cabinet can be best described by reference to the Air Flow Schematic (Figure 2) and the Control Circuit Schematic (Figure 3).

The desired temperature and relative humidity is translated into the equivalent dry and wet bulb temperatures. The air from the test compartments and the accompanying

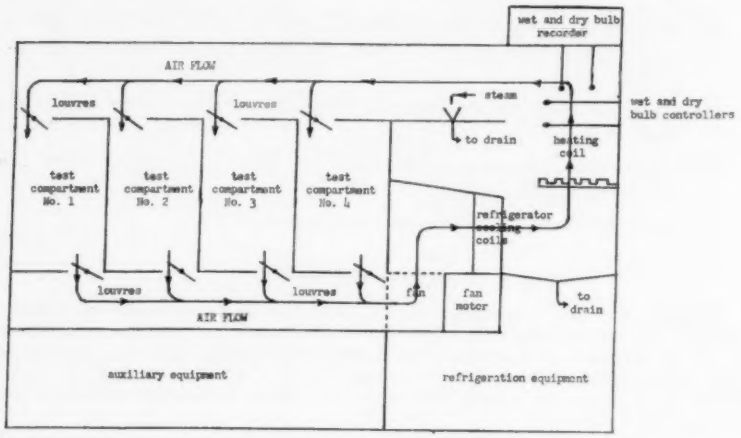
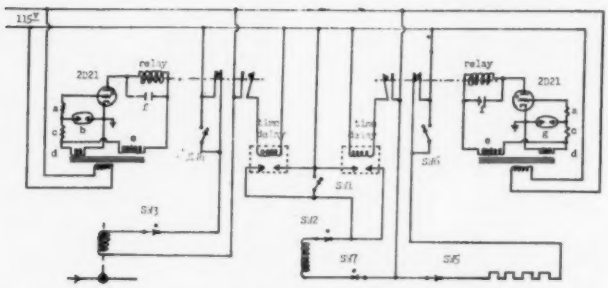



**Figure 2. Air flow schematic.**




| Switch Position | SW 1 | SW 2 | SW 3 | SW 4 | SW 5 | SW 6 | SW 7 |
|---|---|---|---|---|---|---|---|
| Closed | Refrig.Cont.On | Refrig. Auto. | Humid. Auto. | Humid.Cont.On | Heater Auto. | Heater Cont.On | Coil above 32°F. |
| Open | Refrig. Auto. | Refrig.Cont.Off | Humid.Cont.Off | Humid. Auto. | Heater Cont.Off | Heater Auto. | Coil below 32°F. |

**Figure 3. Control circuit schematic.**

control system is recirculated as shown until the desired conditions are attained. This is determined by the wet and dry bulb temperatures which are measured and continuously recorded independent of the control circuit. Once the desired cabinet conditions are obtained, the sensitive controllers and their accessory control circuits maintain these conditions by automatically compensating for any drift, losses, change in ambient conditions, etc.

The air to be conditioned passes over refrigeration coils (Figure 2) whose function is two-fold: cooling and dehumidifying, then over a heating coil which warms and, finally, over a steam jet which humidifies. By a proper split of these opposing adjustments adequate control and adjustment of the conditions are attained. In addition, the outputs of the various components of the conditioning circuit can be adjusted to allow rebalancing of the system when extremes of internal and external load occur.

Details of the control circuit are shown in Figure 3. It consists of a conventional thyratron control circuit in which the grid regains control each cycle and which is biased below cut-off until the preset contacts of the thermoregulators are shorted out by the mercury column.

Examination of the control circuit shows that it can be operated with the heater, the steam jet or the refrigerator on continuously, if desired. The diagram as drawn is with all switches set for fully automatic control.

An additional helpful control is that of the single-action, normally closed thermo-switch on the refrigerator solenoid. Its sensing element is on the cooling coils and is preset at a temperature above 32° F. When the temperature of the coil surface drops to this preset point the refrigeration stops regardless of any signal from the control circuit. The advantages of this additional control are: prevention of frost build-up, avoidance of too-rapid lowering of temperature or humidity and minimization of excessive temperature under-run due to residual coil heat-removal capacity.

**Test compartment section.** The general layout of the test compartments is schematically represented in Figure 4. It consists of a light-tight compartment painted black on the inside through which the conditioned air flows, and which contains the light, mirror, sample pedestal and photographic plate holder to record the outline of the gel at specified intervals. In operation the sample is placed upon the turntable, the turntable is started, a 4 × 5 photographic plate holder is placed in position and the shadow outline of the gel is recorded on the film by turning the light on for the desired length of exposure.

The turntable is used to prevent anomalous results in those cases in which the unmolded gel may sag in a given direction rather than settling perpendicularly. The turntable speed is such to allow a full revolution of the sample during the time exposure on the photographic film. Thus, if the sample has become skewed, it will show in the shape of the outline.

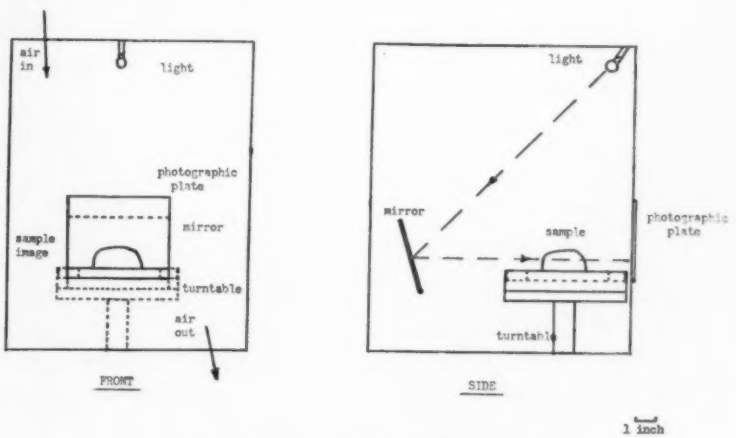



**Figure 4. Schematic of test compartment.**

Previously, samples were positioned in the best profile by hand and if the gel fell other than in the direction of the plane of view it had to be discarded.

The photographic technique to record successive changes in sag and change of shape depends upon a low intensity light source coupled with a very slow photographic film. An ordinary instrument panel light bulb is used as the source and it is connected to a rheostat to permit optimum adjustment of the light intensity. The film is Kodak contrast ortho process film or its equivalent which will permit several 5 to 10 second exposures in this light before complete exposure. In addition, the Graphlock film holder on the front of the compartment is equipped with a ground glass plate and a collapsible light hood which can be used for visual observation of the sample when the film holder is not in place, if desired.

Each compartment can be operated independently of the others with respect to time cycle on the successive shadowgraphs of the sample under study. This is made possible by the individual light and turntable controls for each compartment. Thus, a whole series of measurements on a wide variety of gels can be made at the same time under the same temperature and humidity conditions without adherence to a rigid schedule.

The cabinet can be operated from 40 — 100° F. and provide relative humidities in the range 50 — 95% R.H. at 40° F and 15 — 90% at 100° F. The wet and dry bulb controllers will not vary more than ±0.5° F from their preset control points. The combined tolerances are of the order of ±1° F. The variation in relative humidity corresponding to this precision varies from ±3% at 100° F to ±8% at 40° F.

One other proposed modification is planned for this apparatus. It is a programming device to automatically take the film exposures at preset times. It would consist of a cut-out disc having precut notches at given intervals which would be attached to a clock mechanism and would turn the light on and off at the specified times to complete a full experimental cycle without further attention by the operator.

**Preparation of gels.** A special aluminum mold cup is used which differs slightly from the common household type. It is unflanged, unfluted and has a capacity of 85 ml. The mold can be fitted with a sideboard, usually masking tape, to protect the gels when they are setting in a refrigerated water-bath. The inside of the mold is coated with a silicone emulsion to permit easy removal of the gel from the mold without heat or shaking. The gel solution is poured to the rim of the mold cup and in the case of gelatin desserts, the mold is placed in a refrigerated water-bath at 10° C. for 17 hours.

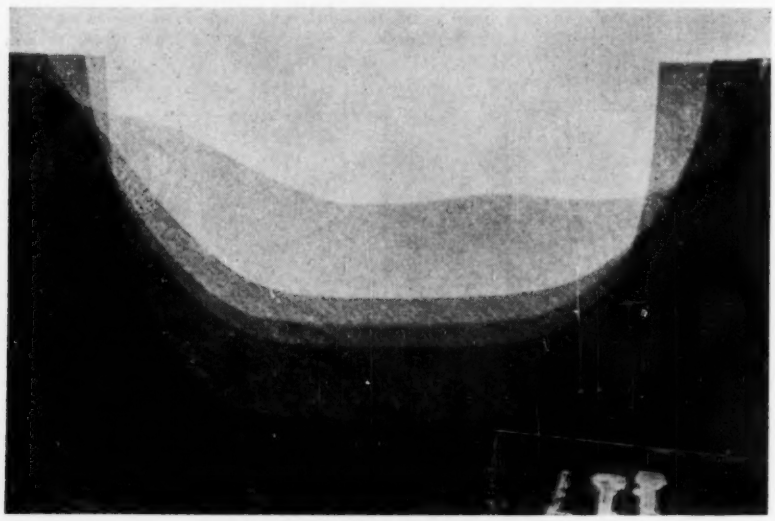


**Figure 5. Typical shadow graph.**

After the gel has set it is removed from the mold cup and placed on a plastic disc. This disc is scored with a series of concentric lines which are used as guides to center the unmolded gel.

**Procedure for measuring sag and melt.** The first exposure is that of the inverted metal mold cup on the plastic disc prior to removal of the gel. This gives a shadow line of the original mold which is used as the reference to measure initial sag. After the initial height of the mold is recorded on the photographic plate, the gel is slipped out and centered on the disc and a second exposure is taken after 2 minutes. Additional exposures of the unmolded gel are taken at fixed time intervals as it melts inside the test cabinet. The intervals are usually 10 minutes for gelatin gels, however, longer intervals are used for slower melting gel systems. A typical multiple exposure on one photographic plate for the sequence of sag and melting is shown in Figure 5. The shadow lines of the metal mold cup and the unmolded gel at various stages of melting can be traced directly from the negative of the photographic plate as shown in Figure 6.

The difference in height between the metal mold and the unmolded gel (Figure 6) measured to the nearest 0.5 millimeter represents initial sag. This difference is directly proportional to the firmness of the gel.

Melt indexes are measured as the difference in height between the unmolded gel after initial sag and after the various time intervals for melting.

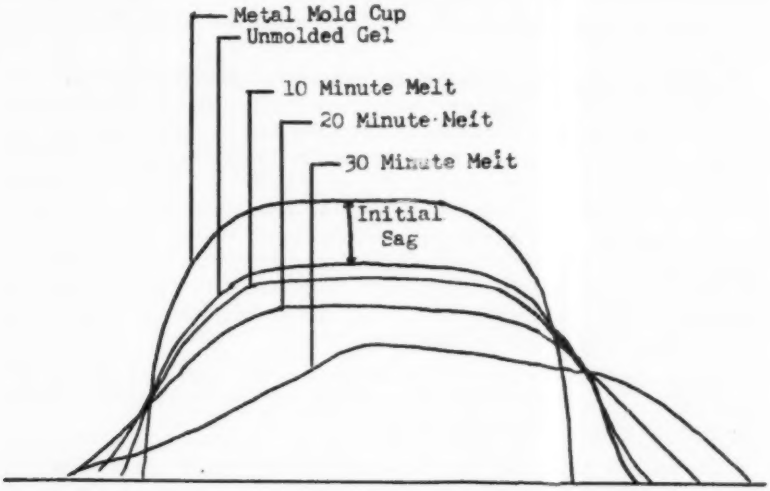



**Figure 6. Initial sag and thermal (melt) sag of unmolded gels.**

## RESULTS AND DISCUSSION

The procedure outlined above provides a method for the evaluation of sag and melt of unmolded gels which is considered by this laboratory to be reliable for experimental work. The results of a typical test of an unmolded gelatin gel repeated on 6 different days are given in Table I.

These data demonstrate the reproductibility that can be realized on different days by the use of this method in the special test cabinet. The variation increases markedly after 30 minutes of melt as shown by the increase in standard deviation, $\sigma$. Part of the day-to-day scatter is due to differences in rate of melt. Best precision is obtained in side-by-side tests in adjoining

**TABLE 1**

**Sag-melt measurements, unmolded gelatin gels repeated tests to measure precision**

| | Initial sag | Decrease in height of unmolded gel at 70 ° F. | | |
|---|---|---|---|---|
| | | 10 min. melt | 20 min. melt | 30 min. melt |
| | *(mm.)* | *(mm.)* | *(mm.)* | *(mm.)* |
| | 9.0 | 2.5 | 3.5 | —— |
| | 9.0 | 1.5 | 4.5 | 12.0 |
| | 8.0 | 1.0 | 2.5 | 5.0 |
| | 8.0 | 1.5 | 5.0 | 13.0 |
| | 9.0 | 1.5 | 4.5 | 9.0 |
| —— | 9.0 | 1.0 | 4.5 | 8.5 |
| $\overline{X}$ | 8.7 | 1.5 | 4.1 | 9.5 |
| $\sigma$ | .45 | .55 | .92 | 3.2 |

compartments of the test cabinet. The variation increases markedly at temperatures above 70° F., or when gels of only moderate firmness are tested. In such cases the gels soften rapidly and severe distortion of the shape of the unmolded gel occurs.

**Effect of temperature on gelatin gels.** The procedure outlined was to determine a critical temperature to evaluate gelatin gels. A series of molds were prepared with two widely different gelatin formulations. The gels were set in a water-bath to a constant firmness and then removed and measured in the test cabinet at temperatures of 50°, 60°, 70°, 80° and 90° F. The results for two gelatin gels are plotted in Figure 7 to show the decrease in height of the unmolded gels after standing 30 minutes. It can be seen there is a slight change between 50° and 60° for both gels. At 70° F there is some indication of difference in melting rate, however, at 75° and 80° the differences are pronounced. Gel I undergoes 10 mm decrease in height after 30 minutes at 75° F compared to 4 mm for Gel II.

Similar experiments were run at different relative humidities in the temperature range of 50-80° F. No systematic differences in sag or melting could be attributed to relative humidity.

The method which employs the use of the test cabinet can be adapted to many applications in experimental and control work. The method can be used to clearly differentiate between sag and melt. It can also be used to determine melting temperatures of different gel systems. The effect of cycling is another important consideration in the evaluation of gels, especially for the difference in temperature between a consumer's refrigerator and the dinner table. The study of unmolded gels under different temperature cycles can be readily accomplished with this instrument.

The numerical ratings obtained from the measurements permit quantitative evaluation in the study of the many factors and their inter-relations that influence the setting and melting properties of gel systems. The study of these factors in terms of the performance of unmolded gels is a critical and a practical evaluation that is directly related to the acceptance of the product by the consumer.

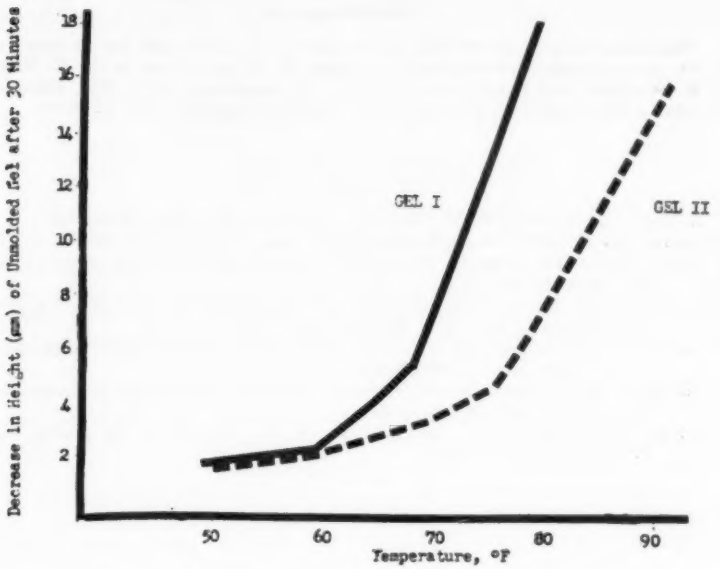



**Figure 7. Effect of temperature on melt of gelatin gels.**

## SUMMARY

The study of gel systems for dessert products has attracted attention to the relaxation and melting characteristics of unmolded gels. Pectin, alginate gels and others of the non-melting types can be characterized by relaxation or sag measurements only. Gelatin gels exhibit both sag and melt at temperatures above 70° F.

The sag and change of unmolded gels can be measured by obtaining a series of shadowgraphs on a photographic plate. Graphic translations of the shadow lines enable objective measurements which are useful to study melting and relaxation characteristics of gels.

A special test cabinet was designed for these measurements. It consisted of a series of compartments containing sample platform and a light source to cast the shadow of the gel on a photographic plate. The design includes provisions for casting a succession of shadows on the same photographic plate without disturbing the sample or opening the compartment. Each compartment can be operated independently with respect to time cycle. In addition, the measurements can be made over a range of temperatures and relative humidities.

Many applications are possible by the method in conjunction with the test cabinet to evaluate gel systems. The separate measurements of sag and melt at different temperatures can be used to give a complete description of the performance of unmolded gels in the consumer's home from the refrigerator to the dinner table.

**Acknowledgments**

The authors wish to acknowledge the assistance of J. M. Reynolds for his contributions in the early planning of the method and design of the test cabinet to K. R. Newman, C. M. Woodcock, and I. I. Litman for advice and suggestions and to Wm. Johnson and Associates, who constructed the test cabinet, for their cooperation and assistance.

## LITERATURE CITED

1. Baker, G. L., Goodwin, M. W. Delaware Agr. Expt. Sta. *Bull. 234* (1941).
2. Bender, W. A. Grading pectin in sugar jellies. *Anal. Chem.*, **21**, 408 (1949).
3. Cox, R. E., and Higby, R. H. A better way to determine the jellying power of pectins. *Food Inds.*, **16**, 441 (1944).
4. Lampitt, L. H., Money, R. W. Pectin Gels I A method of measurement of the strength of pectin gels. *J. Soc. Chem. Ind.*, **56**, 290T (1937).
5. Lampitt, L. H., and Morris, M. E. G. Measurement of the strength of gelatin gels. *J. Soc. Chem. Ind.*, **53**, 179 (1934).
6. Lockwood, H. C., and Hayes, R. S. A new method for testing agar and gelatin jellies, *J. Soc. Chem. Ind.*, **50**, 145T (1931).
7. Owens, H. W., Porter, O., and Maclay, W. D. New device for grading pectins. *Food Inds.*, **19**, 606 (1947).

# HEAT TRANSFER COEFFICIENTS IN STRAIGHT TUBES FOR PSEUDOPLASTIC FOOD MATERIALS IN STREAMLINE FLOW[a,b]

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(Manuscript received May 31, 1958)

In almost every phase of the industrial processing of food, the physical factors of heating and cooling are involved at some point in the procedure. The need for improving heat transfer has led to the development of much specialized equipment. The level of viscosity of many fluid food products is sufficiently high to prevent turbulence under practical rates of flow, and therefore, the generally low heat transfer coefficients associated with streamline flow are common in food processing equipment.

For Newtonian liquids in streamline flow (laminar), equations have been developed which are employed in the design and operation of heat exchangers *(3)*. However, many food materials are plastic or pseudoplastic and the equations developed for Newtonian materials are not applicable to this type of fluid. A pseudoplastic fluid is defined as one for which there is no yield value and for which the ratio of shear stress to shear rate decreases with increasing shear stress or rate. A plastic fluid has a yield value (e.g., mayonnaise).

Methods for determining the flow characteristics of pseudoplastic materials are reported in the literature, of which reference *(1)* is an example. This paper is concerned with an investigation of the heat transfer behavior of fluids in streamline flow that are predominantly pseudoplastic in character.

## DEVELOPMENT OF MATHEMATICAL EXPRESSIONS

The rate of heat transfer to a fluid flowing through a tube can be expressed by the following equation which, though not in conventional form, is well suited to studies on the relatively viscous materials considered in this work wherein both the outside local coefficients and the wall conductance are very high compared to the local inside coefficient which is sought:

$$q = wc_p (T_2 - T_1) = U_o^1 A_o \frac{(T_w - T_1) - (T_w - T_2)}{2.3 \log \left( \frac{T_w - T_1}{T_w - T_2} \right)} \qquad (1)$$

and

$$\frac{1}{U_o^1} = \frac{\times A_o}{K_w A_m} = \frac{A_o}{A_i h_i} \qquad (2)$$

where: $q$ = rate of heat transfer (Btu's/hr.)
$W$ = flow rate (lbs./hr.)
$C_p$ = specific heat (Btu's/lb. — °F)
$T_1$ = inlet temperature of fluid (°F)
$T_2$ = outlet temperature of fluid (°F)

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[a] Contribution No. 337 from the Department of Food Technology.
[b] Presented at the Eighteenth Annual Meeting of the Institute of Food Technologists, Chicago, May 27, 1958.

$T_w$ = average outside wall temperature of tube (°F)
$A_o$ = outside heat exchange area (sq. ft.)
$A_1$ = inside heat exchange area (sq. ft.)
$A_m$ = mean heat exchange area (sq. ft.)
$U_o^{1*}$ = heat transfer coefficient $\left(\frac{\text{Btu}}{\text{(hr.) (sq. ft.) (°F)}}\right)$
X = tube wall thickness (ft.)
$K_w$ = thermal conductivity of tube $\left(\frac{\text{Btu}}{\text{(hr.) (sq. ft.) (°F)}}\right)$
$h_1$ = local heat transfer coefficient of fluid within tube

The problem usually is to calculate the fluid outlet temperature for a given set of conditions or possibly the conditions necessary to attain a certain outlet temperature.

The term which is the most difficult to determine is the heat transfer coefficient associated with the pseudo-plastic fluid which, in this case, is within the tube.

Sieder and Tate *(6)* correlated heat transfer data for viscous Newtonian fluids in forced laminar flow using the empirical equation:

$$\frac{hD}{k} = 2.0 \left(\frac{WC_p}{k_L}\right)^{.33} \left(\frac{\mu_a}{\mu_w}\right)^{.14} \tag{3}$$

where: h = heat transfer coefficient
D = tube diameter
K = thermal conductivity of fluid
W = mass rate of flow
$C_p$ = specific heat of fluid
$\mu$ = density of fluid
$\mu_a$ = viscosity (bulk average temperature)
$\mu_w$ = viscosity (wall temperature)

The dimensionless group $\left(\frac{\mu_w}{\mu_a}\right)$ was required to correct for the influence of temperature on viscosity and consequently on the flow pattern.

Equation *(3)* was suggested for use with viscous materials where $\frac{\mu}{p}$ was large and natural convection currents small *(4)*.

*Pseudoplastic fluids*

It is possible to use the same approach to determine an empirical equation for pseudoplastic fluids which expresses the heat transfer coefficient "h" as a function of the other variables involved.

Scott Blair *(5)* and Merrill *(1)*, to cite only two among many, found it possible to characterize pseudoplastic fluids by the equation

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* $U_o^1$ includes the following resistances to heat transfer: (a) tube wall, (b) fluid within tube. The reason for having a coefficient $U_o^1$ as defined will become evident from the description of the experimental apparatus.

**TABLE 1**

**Comparison of calculated values of h with experimental values for various substances**

| Material | Type of heat transfer | h exp. | h calc. | % diff. from h exp. | Fluid temp. range (° F) | Tube wall temp. (° F) |
|---|---|---|---|---|---|---|
| Ammonium alginate (3.37%) S = .477 | heating | 22.5 | 23.4 | +6 | ( 74.8- 89.6) | 96.2 |
| | heating | 64.0 | 55.0 | −10 | (178.8-185.7) | 213.5 |
| | heating | 41.4 | 38.6 | −9 | (101.6-108.2) | 119.4 |
| | heating | 36.8 | 37.6 | +2 | (112.0-150.0) | 210.3 |
| Ammonium alginate (3.30%) S = .534 | cooling | 36.2 | 42.7 | +15 | (113.8-106.9) | 67.0 |
| | cooling | 24.8 | 30.5 | +18 | (113.0-99.2) | 65.7 |
| | heating | 45.5 | 36.0 | −20 | (106.5-162.1) | 214.0 |
| | heating | 143.0 | 157.5 | +9 | (102.0-146.6) | 216.6 |
| Ammonium alginate (2.00%) | heating | 28.2 | 32.6 | +13 | ( 84.2-103.0) | 138.5 |
| | heating | 59.7 | 60.0 | 0 | (121.8-135.4) | 209.6 |
| | heating | 54.6 | 58.0 | +5 | ( 90.9- 97.4) | 135.5 |
| | heating | 41.7 | 34.0 | −18 | (109.4-162.1) | 214.8 |
| | cooling | 42.0 | 32.0 | −24 | (112.2- 91.3) | 70.8 |
| Applesauce S = .645 | cooling | 46.6 | 43.4 | −6.0 | (122.5-108.6) | 70.2 |
| | heating | 53.5 | 47.5 | −11.0 | ( 87.6-101.7) | 135.2 |
| Banana puree S = .458 | heating | 62.0 | 49.0 | −20.0 | (112.0-141.3) | 208.0 |
| | cooling | 38.4 | 36.0 | −6.5 | (112.2-100.4) | 67.9 |
| | heating | 124.0 | 144.0 | +14.0 | ( 77.6- 99.2) | 132.2 |
| | heating | 230.0 | 184.0 | −20.0 | (115.5-140.1) | 212.6 |
| | cooling | 161.0 | 185.0 | −13.0 | (116.0-106.6) | 68.2 |
| | cooling | 118.0 | 133.0 | +10.0 | (115.6- 97.7) | 64.8 |
| | heating | 140.0 | 143.0 | +2.0 | ( 77.5- 99.8) | 131.5 |
| | heating | 161.0 | 159.0 | −1.0 | (102.8-155.1) | 213.8 |
| Motor oil | heating | 23.3 | 21.5 | −8.0 | ( 88.1- 93.0) | 135.9 |

$$\tau = b \left(\frac{d\gamma}{d\theta}\right)^s \tag{4}$$

where: $\tau$ = shear stress

$b$ = proportionality factor

$\frac{d\gamma}{d\theta}$ = rate of shear

$s$ = constant

Equation *(4)* is not valid at zero rate of shear. The lowest rate of shear which still conforms to equation *(4)* depends on the fluid. Ammonium alginate solutions can be correlated by equation *(4)* with single values of b and s over the range of 100 to 20,000 $sec^{-1}$, and by only slightly different values over the range of 0.1 to 42 $sec^{-1}$ of shear rate.

For pseudoplastic materials "s" theoretically can vary between the values of 0 and 1. For polymer solutions that *have no yield value* a value of "s" less than 0.4 has not been reported in the literature as yet. For solutions of free-draining polymers the variation of "s" with temperature over limited ranges of temperature in the fluid studied herein, is shown in Table 2. From the

**TABLE 2**

**Values of the pseudoplasticity constant s as a function of temperature for various materials**

| Material | Temp. ° F | Value of s | Value of $\tau$ at 1000 1/sec $= \frac{d\gamma}{d\phi}$ (dynes/cm²) × $10^{-3}$ |
|---|---|---|---|
| Ammonium alginate (3.37%) | 76 | .500 | 3.70 |
| | 90 | .464 | 3.70 |
| | 105 | .486 | 2.90 |
| | 113 | .487 | 3.00 |
| | 130 | .450 | 2.7 |
| Ammonium alginate (2.00%) | 77 | .611 | 1.25 |
| | 91 | .605 | 1.15 |
| | 107 | .592 | 1.05 |
| | 115 | .596 | 0.98 |
| | 131 | .625 | 0.75 |
| Applesauce | 72 | .645 | .37 |
| | 90 | .645 | .33 |
| | 105 | .640 | .28 |
| | 121 | .650 | .22 |
| Banana purée | 68 | .460 | 1.75 |
| | 77 | .477 | 1.40 |
| | 107 | .486 | 1.35 |
| | 120 | .478 | 1.10 |
| Ammonium alginate | 79 | .535 | 2.90 |
| | 95 | .544 | 2.80 |
| | 101 | .530 | 2.70 |
| | 108 | .525 | 2.25 |
| | 120 | .535 | 2.20 |

data shown therein, it appears reasonable to use a single average value of "s" over a range of 50° F for purposes of the correlation described below. When equation *(4)* is fitted to fluids in the upper range of shear rates that have a yield value (i.e., certain kinds of plastic fluids), apparent values of "s" considerably lower than 0.4 can result.

The proportionality factor "b" appears to vary with temperature in a manner analagous to the variation of viscosity with temperature. The plot of the log b vs. the reciprocal of the absolute temperatures appears to be a straight line (Merrill *(1)* ). This is the basis of the correlation shown in Figure 1.

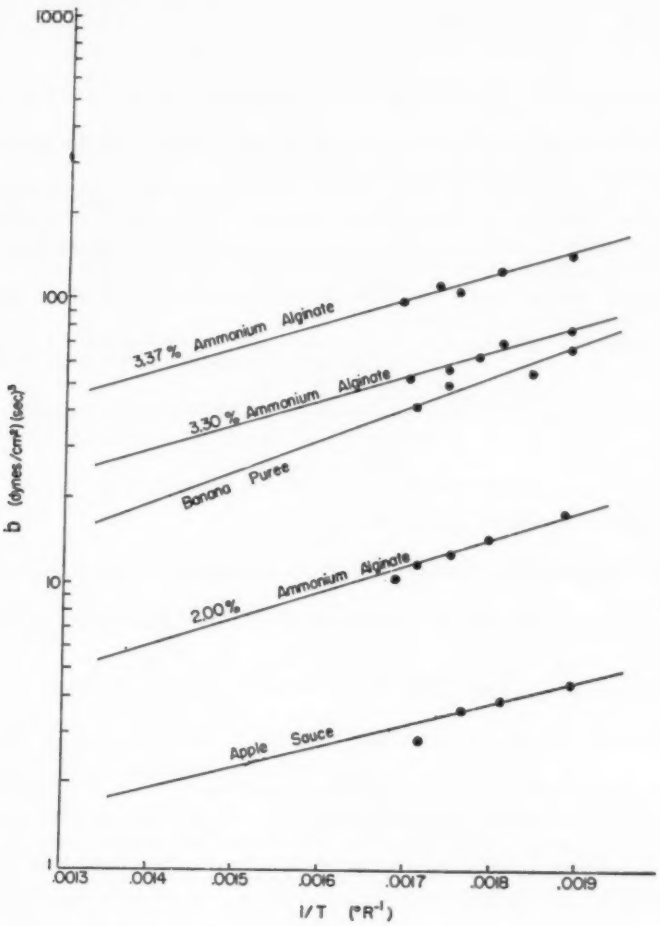



**Figure 1. Variation of log proportionality factor, b with the reciprocal of absolute temperature for various materials.**

When $s = 1$ equation *(4)* reduces to the form which characterizes a Newtonian material and $b = \mu$.

The term $\frac{\tau}{\left(\frac{d\gamma}{d\theta}\right)}$ is defined as the apparent viscosity and is a function of $\tau$ or $\frac{d\gamma}{d\theta}$.

One method of correlating data for laminar flow heat transfer in pseudoplastic liquids is suggested by substituting $\left(\tau/\frac{d\gamma}{d\theta}\right)_a$ for $\mu_a$ and $\left(\tau/\frac{d\gamma}{d\theta}\right)_w$ for $\mu_w$ in equation *(4)*.

The purpose of $\frac{\mu_a}{\mu_w}$ in equation *(3)* is to correct for variations in viscosity due to temperature variations along the tube radius and the subsequent deviation of the velocity profile from a parabola.

The velocity profile of a Newtonian fluid flowing in isothermal flow is parabolic. However, a pseudoplastic material flowing in a tube does not exhibit a parabolic velocity profile even in isothermal flow. It appears that it might be possible to correct for the deviation of velocity profile from a parabola due to pseudoplasticity as well as temperature variation along the tube radius by employing the ratio:

$$\left[\frac{\left(\tau/\frac{d\gamma}{d\theta}_a\right)}{\left(\tau/\frac{d\gamma}{d\theta}_w\right)}\right]^{.14}$$

since this is somewhat analagous to $\left(\frac{\mu_a}{\mu_w}\right)^{.14}$

In considering the isothermal flow of a pseudoplastic fluid such as a 3.3% ammonium alginate solution, it becomes apparent that $\left(\tau/\frac{d\gamma}{d\theta}\right)$ varies with the velocity of the fluid. This is demonstrated by the curve $\tau$ vs. $\frac{d\gamma}{d\theta}$ (Figure 2). At low rates of shear (100 $sec^{-1}$), the ratio $\tau/\frac{d\gamma}{d\theta}$ is relatively high ($1000/100 = 10$ poises), whereas at 8000 $sec^{-1}$ it is $10{,}000/8000 = 1.25$ poises. Thus, under a uniform shear rate of 100 $sec^{-1}$, the particular pseudoplastic fluid would correspond to a Newtonian fluid with a viscosity of 10 poises, whereas at 8000 $sec^{-1}$ it would behave as a Newtonian fluid with a viscosity of 1.25 poises.

The variation of $\left(\tau/\frac{}{d\theta}\right)$ along the radius may be determined from

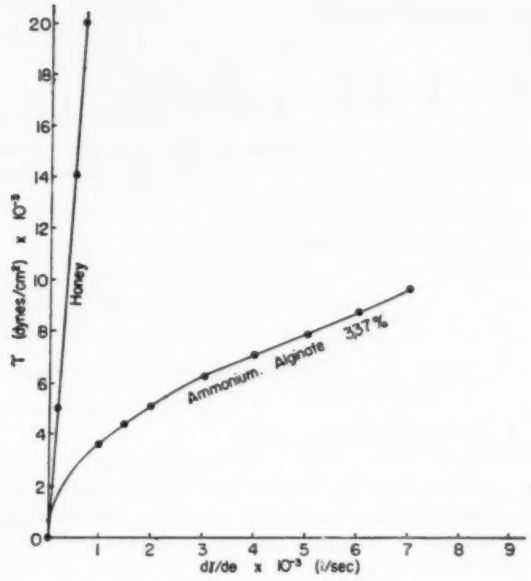



**Figure 2. Comparison of Newtonian honey with pseudoplastic ammonium alginate on shear stress rate of shear diagrams.**

a consideration of forces acting on an annular cylinder fluid with a differential thickness as the fluid flows through a straight tube.

$$\Delta P \pi R^2 = \tau(2\pi R \Delta L) \tag{5}$$

In the case of pseudoplastic fluid that followed the empirical relation $\tau = b\left(\frac{d\gamma}{d\theta}\right)^s = b\left(\frac{dV}{dR}\right)^s$. Merrill *(1)* found that substitution of this relation in equation *(5)* followed by integration, yielded:

$$V = \left(\frac{4\Delta P/\Delta L}{2b}\right)^{1/s} \cdot \left(\frac{Rw^{(1/s+1)} - R^{(1/s+1)}}{1/s+1}\right) \tag{6}$$

where: $R_w$ = tube radius
$V$ = point velocity

From this the volumetric rate of flow was found to be

$$Q = \frac{\pi}{1/s+3} \cdot \left(\frac{\Delta P/\Delta L}{2b}\right)^{1/s} \cdot R_w^{(1/s+3)} \tag{7}$$

Thus, from equation *(7)* $\tau = \left(\frac{\Delta P}{\Delta L(2)}\right)^R$ and by rearrangement of equation *(4)* $\frac{d\gamma}{d\theta} = \left(\frac{\tau}{b}\right)^{1/s}$. For straight tubes $\frac{d\gamma}{d\theta} = \frac{dV}{dR}$ where $V$ = velocity

and R = radial distance from axis.
Therefore:

$$\left(\tau = \frac{d\gamma}{d\theta}\right) = \left(\tau / \frac{dV}{dR}\right) = \frac{(\Delta P/2\Delta L)^R}{\left[(\Delta P/2\Delta L)\left(\frac{R}{b}\right)\right]}^{1/n} \tag{8}$$

Equation *(7)* may be used to calculate $\Delta P/\Delta L$ for a given volumetric rate of flow, and equation *(8)* is an expression for the radial distribution of

* $\left(\frac{d\gamma}{d\theta}\right) = \left(\frac{dV}{dR}\right)$ in straight tubes

$\left(\tau / \frac{dV}{dR}\right)$. If $\Delta P/\Delta L$ has the dimensions of dynes/cm$^3$ and R, in cm., then $\left(\tau / \frac{dV}{dR}\right)$ can be expressed in poises.

In order to evaluate the bulk average ratio it was decided to employ the average of the fluid flowing, since in equation *(3)* is evaluated at a temperature associated with the velocity average of the fluid, e.g., the "mixing-cup" temperature, defined as:

$$Tmc = \frac{\int_0^{R_w} 2\pi RdRVT}{\int_0^{R_w} 2\pi RdRV} \tag{9}$$

where V is the point velocity and T the point value of temperature at any radial distance R.

Mathematically, the bulk average ratio of $\left(\tau / \frac{dV}{dR}\right)_a$ may be expressed by

$$\left(\tau / \frac{dV}{dR}\right)_a \int_0^{R_w} 2VRdR = \int_0^{R_w} 2\left(\tau / \frac{dV}{dR}\right) VRdR \tag{10}$$

Substituting from equations *(8)* and *(9)* and integrating

$$\left(\tau / \frac{dV}{dR}\right)_a = \left[\frac{2}{(\Delta P/\Delta L)_a}\right]^{1/n+1} b_a \left[\frac{(3+1/n)}{(3-1/n)(2)(R_w^{(1/n-1)})}\right] \tag{11}$$

From equation *(8)*

$$\left(\tau \frac{dV}{dR}\right)_w = \left[\frac{2}{(\Delta P/\Delta L)_w}\right]^{1/s-1} b_w^{1/s} \left(\frac{1}{R_w}\right)^{1/s-1} \qquad (12)$$

Combining equations *(11)* and *(12)*

$$\frac{\left(\tau/\frac{dV}{dR}\right)_a}{\left(\tau/\frac{dV}{dR}\right)_w} = \frac{b_a}{b_w}\left[\frac{(3+1/s)}{(3-1/s)\ (2)}\right] \qquad (13)$$

A means of correlating heat transfer data for pseudoplastic liquids in laminar flow is suggested by equation *(3)*.

By substituting equation *(13)* in equation *(3)* this becomes

$$\frac{hD}{K} = 2\left(\frac{WC_p}{K_L}\right)^{.33}\left[\left(\frac{b_a}{b_w}\right)\left(\frac{(3+1/s)}{(3-1/s)\ (2)}\right)\right]^{.14} \qquad (14)$$

In the analagous Sieder-Tate expression for Newtonian fluids, the viscosity $\mu_a$ was evaluated at the bulk average temperature, and $\mu_w$ at the wall temperature.

To continue the analogy, $b_a$ was evaluated at the bulk average temperature, $b_w$ at the wall temperature, while as previously mentioned, "s" appears to be essentially independent of temperature.

The value of $\dfrac{\left(\tau/\frac{dV}{dR}\right)_a}{\left(\tau/\frac{dV}{dR}\right)_w}$ may be solved graphically using equations *(10)* and *(12)*. It is noted from equation *(14)* that when $s = 1/3$, $\dfrac{\left(\tau/\frac{dV}{dR}\right)_a}{\left(\tau/\frac{dV}{dR}\right)_w}$ becomes infinite. This is a peculiarity which, resulting from the integration, is not encountered if the graphical solution of equation *(13)* is used and a small central core of fluid is arbitrarily neglected.

It was explained previously that equation *(4)*, which underlies the above analysis is never valid at $\tau = 0$ and therefore at $R = 0$. To disregard the equation close to the axis is not a simplifying assumption, but rather an absolute necessity in the light of physical reality. In contrast, equations *(6)* and *(7)* may be integrated from $R = 0$ to $R_w$ after substitution of equation *(4)*, provided that equation *(4)* fits the rheology of the fluid beyond, say, $R = .01\ R_w$. This particular integration is not sensitive to the error in the $\tau$ vs. $\dfrac{d\gamma}{d\theta}$ relation inherent in equation *(4)* near $\dfrac{d\gamma}{d\theta} = 0$

In the graphical integration a plot is made of

$\left(\frac{2}{\Delta P/\Delta L}\right)^{1/s-1} b^{1/s}\left[\left(R_w^{(1/s+1)}\right)\left(R^{2-1/s}\right) - R^3\right]$ vs. R between the limits of $R = 0$ and $R = R_w$. The area under the resulting curve is related to $\left(\tau/\frac{dV}{dR}\right)_a$ (see Fig. 3). If "s" is less than 0.5, at $R = 0$ the curve goes

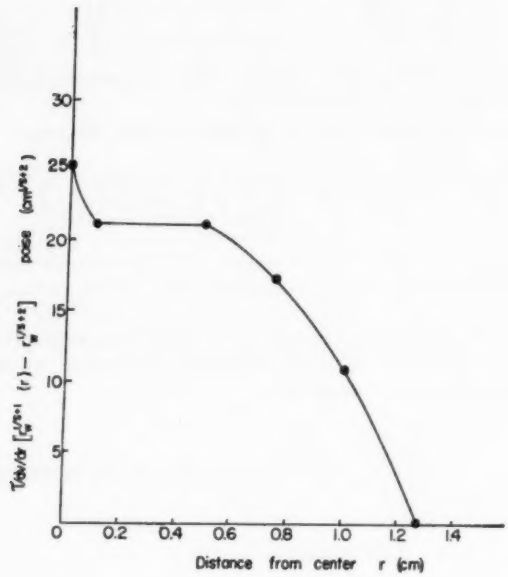



**Figure 3.** $\tau\frac{dV}{dR}\left[r_w^{1/s+1} - r^{1/s+2}\right]dr$ **as a function of r for evaluating** $\left(\int_0^{r_w} \tau\frac{dV}{dR}\left[r_w^{1/s+1}(r) - r^{1/s+2}\right]dr\right.$

to infinity. To overcome this as previously explained, a lower limit of $R = .01\ R_w$ is chosen and the curve graphically extrapolated to $R = 0$. Physically, this assumes that neglecting the volume associated with regions close to the center will not affect the overall average appreciably.

It is noted that both the analytical and graphical solution checked for cases where $s = 0.477$ and $s = 0.458$.

## EXPERIMENTAL

### A. The recycling heat exchange system

The experimental apparatus used for determining the experimental heat transfer coefficient is illustrated in Figure 5.

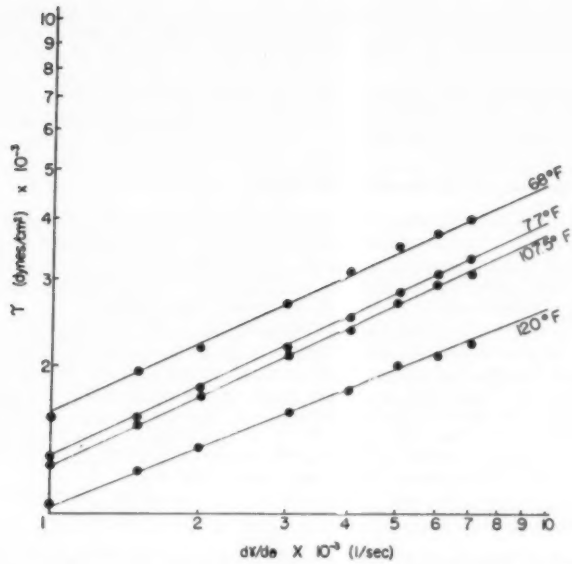
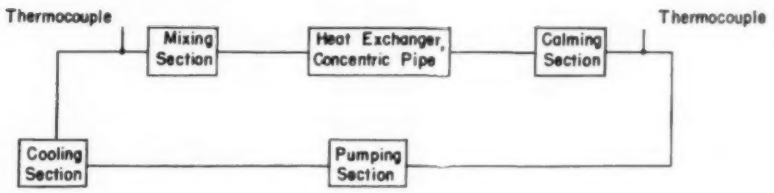



**Figure 4. Variation of log shear stress with log rate of shear for banana puree (S = 0.458) at various temperatures.**




Heat Exchange System

**Figure 5. Arrangement of heat exchange system.**

The fluid is pumped through a calming section, as used by Sieder and Tate *(6)*, before entering the heat exchanger. Immediately after leaving the heat exchanger, the fluid passes through a baffling system which thoroughly mixes it. The exit temperature of the fluid is measured after it is mixed by the baffles.

The fluid is then passed through a cooling section and then back to the pump to be recycled.

The rate of fluid flow was measured by diverting the flow from the cooling section to a bucket on a balance. The weight for a given time interval measured by a timer was determined. The mass rate of flow was assumed to be constant with time since a chain drive Vari-drive motor was used to power the pump.

Thermocouples placed at various points along the tube wall in the heat exchanger permitted the determination of the tube temperature.

When inlet and outlet temperatures of the fluid, the outside wall temperature, and the mass rate of flow were known, it was possible to determine the heat transfer coefficient from equations *(1)* and *(2)*. Tubes ranging in inside diameter from .25" to 1.00" were employed.

The experimental heat transfer coefficients were checked with those calculated from equation *(14)*. In order to employ equation *(14)*, it is necessary to know the heat capacity and thermal conductivity of the fluid.

### B. Determination of thermal conductivity

A thermal conductivity measuring device was constructed from two pieces of Armco Iron, a commercially pure iron, each 2.00 inches in diameter and one foot long.

To one of these pieces was attached a machined bakelite ring 0.001 inches high and two inches in diameter. Armco iron was used because it is pure and data on the thermal conductivity of this iron as a function of temperature was available.

The iron with the bakelite ring was supported vertically in a ring stand and formed the bottom of the measuring device. The material to be tested was placed in the cell formed by the bakelite ring and the machined faces of Armco iron. The machined face of the other piece of Armco iron was placed on top of the bakelite ring to form the top of the cell and the top section of the measuring device. A ridge in the bakelite helped support the top section which was also supported by a ring stand. Copper-Constantan thermocouples were located at various points along the iron cylinders. The top section of iron was heated while the bottom section of iron was cooled (Fig. 5).

### C. Viscometry

The Merrill viscometer *(2)* was used to determine the shear stress rate of shear relations of the fluids used in this study.

In general, the accuracy of the data obtained from this viscometer was $\pm 5\%$ above a shear rate of $200^{-1}$. The shear rate at wall in these experiments varied from approxi-

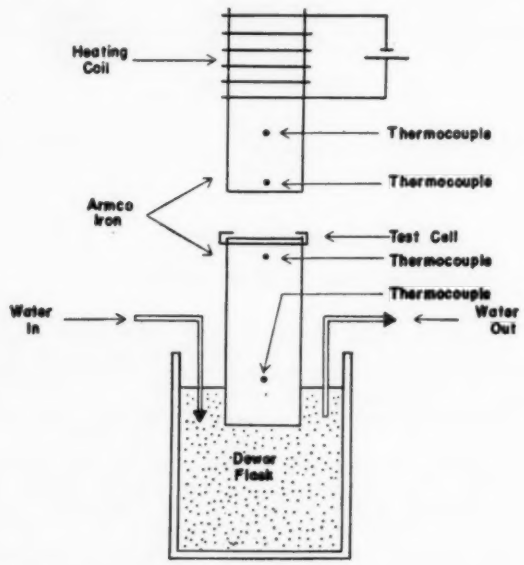



**Figure 6. Thermal conductivity measuring device.**

mately 50 to 1500 $sec^{-1}$. However, cases in which the shear rate at the wall was less than 200 $sec^{-1}$ did not result in any greater percentage error in the calculation of the heat transfer coefficient than did those cases where the rate of shear at the wall was greater than 200 $sec^{-1}$. This indicates that the precision of viscometry information associated with rates of shear at the wall between 50 and 200 $sec^{-1}$ did not appreciably affect the precision of the heat transfer coefficient as calculated by equation *(14)*.

The viscometer is provided with internal circulation of water from a thermostatic bath which controls temperature at both the cylindrical surfaces. Therefore, measurement of the fluid at various temperature levels is relatively simple to achieve.

## RESULTS

Equation *(14)* was checked experimentally with various pseudoplastic materials such as banana puree, apple sauce, 3.37% ammonium alginate solution and 2.00% ammonium alginate solution (see Fig. 4 and Table II).

It may be reasonably argued that banana and applesauce may have a very small yield value as it is associated with a plastic material. However, the experimentally obtained curve of $\tau$ vs. $\delta\gamma/\delta\theta$ over a wide range shows the unmistakable curvature of the pseudoplastic fluid. It is tentatively concluded that their low yield is of negligible effect under practical conditions of flow.

The results using equation *(14)* indicate that the calculated values of "h" agree within $\pm$ 24% with the experimental values (Table I). Perry, J. *(4)* noted that for analagous Sieder-Tate equation for Newtonian materials (or equation *(14)* when $s = 1$), the agreement is in the order of $\pm$ 15. Therefore, agreement of the predicted values with experimental data within $\pm$ 24% should be considered satisfactory.

Equation *(14)* has been found to be satisfactory for calculating heat transfer coefficients during the cooling as well as the heating process.

## SUMMARY

An equation has been developed for determining the heat transfer coefficients of pseudoplastic fluids in straight tubes. The calculated results agreed within 24% with experimental values.

### Acknowledgment

The authors are appreciative of financial assistance in the form of grants-in-aid from the following organizations which made a part of this program possible: Armour and Company, Carnation Company, The Nestle Company, and Oscar Mayer and Company.

## LITERATURE CITED

1. Merrill, E. W. Pseudoplastic flow: Viscometry, Correlations of shear stress vs. stress rate and predictions of laminar flow in tubes. *J. Colloid Sci.*, **11**, 7-14 (1956).
2. Merrill, E. W. A coaxial cylinder viscometer for non-Newtonian fluids. *I.S.A. Journal*, **3**, (4) 124 (1956).
3. McAdams, W. H. *Heat Transmission* (3rd ed.) McGraw-Hill Cook Company, New York, pp. 229-239 (1954).
4. Perry, J. H. *Chemical Engineers' Handbook*, McGraw-Hill Book Co., New York, p. 474 (1950); *loc. cit.*, p. 237.
5. Scott-Blair, G. H. *Introduction to Industrial Rheology*, 1938. J. and A. Churchill, London, cited from *Advances in Chemical Engineering*, Drew T. R. and Hoopes, J. W., p. 85 (1956).
6. Sieder, E. N. and Tate, G. E. Heat transfer and pressure drop of liquids in tubes. *Ind. Eng. Chem.*, **28**, 1429 (1936).

# FOLIC ACID METABOLISM IN GERMINATING SEEDS

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(Manuscript received August 6, 1958)

It was reported earlier from this laboratory that folic acid (FA) contents of pulses *(Leguminous seeds)* decreased during germination *(2)* although other vitamins such as ascorbic acid *(11)*, thiamine *(6)*, riboflavin *(12)*, carotene *(3)*, choline *(4)*, tocopherol *(5)*, biotin and niacin *(2)*, pyridoxine, inositol and vitamin K *(1)* and vitamin $B_{12}$ *(15)* increased during germination of the seeds. It was of interest to study the factors which led to the diminution of FA contents of germinated seeds.

It has now been established that FA is converted in the tissues into citrovorum factor (CF) *(14, 17)* and ascorbic acid augments this conversion *(13)*. As ascorbic acid contents of germinated pulses increased during germination it was possible that the diminution of FA might be due to its conversion into CF by the ascorbic acid which is synthetised by the pulse during germination. Folic acid is involved in the synthesis of nucleic acid *(9, 10)*. Ribonucleic acid (RNA) and CF contents of pulses were, therefore, determined both before and after germination. Preliminary investigation indicated the absence of desoxyribonucleic acid in ungerminated and germinated seeds.

## EXPERIMENTAL

**Germination of the seeds.** Nearly 1 g. of clean, dry and healthy looking seeds were weighed and kept in sterilized petri dishes. Two cc. of glass distilled water was added on the first day and 1 cc. on subsequent days and the dishes were kept away from direct sun light. Seeds were allowed to germinate at room temperature (30° C.) for 2 and 4 days.

**Estimation of CF.** Powdered ungerminated as well as the germinated seeds were macerated with water and transferred quantitatively into 50 cc. conical flasks, 5 cc. of 0.1 M acetate buffer of pH 4.5 added to each of the flasks, autoclaved for 15 minutes at 15 pound pressure, cooled, incubated with 2.5 cc. hog kidney enzyme preparation under toluene for 24 hours at 37° C., autoclaved again for 5 minutes at 15 pound pressure, cooled,

**TABLE 1**

**Citrovorum factor contents of pulses (μ per 100 g.)**
**Figures are expressed in terms of the wet weight of the original seed taken for germination**

| Local name | Botanical name | Ungerminated | After germination | |
|---|---|---|---|---|
| | | | 2 days | 4 days |
| Arhar ..... | *Cajunas indicus* | 27 ± 0.1 * | 9.7 ± 0.1 | 9.5 ± 0.1 |
| Chhola ..... | *Cicer arietinum* | 124 ± 0.1 | 21.5 ± 1.6 | 24.5 ± 1.3 |
| Khesari ..... | *Lathyrus sativus* | 55 ± 3.1 | 25.5 ± 0.1 | 19.0 ± 2.3 |
| Mung ..... | *Phaseolus radiatus* | 48 ± 3.3 | 13.7 ± 1.5 | 16.7 ± 1.3 |
| Mator ..... | *Pisum sativum* | 17 ± 1.2 | 9.7 ± 0.3 | 14.2 ± 0.7 |
| Kalaii ..... | *Phaseolus mungo* | 100 ± 3.5 | 58.7 ± 1.1 | 41.0 ± 1.8 |
| Musur ..... | *Lens esculenta* | 47 ± 2.0 | 40.7 ± 1.0 | 46.2 ± 2.0 |

* Mean ± Standard Error.

adjusted to pH 7, volume made up to 50 cc. with water and filtered. In the filtrate CF was determined using *Leuconstoc citrovorum 8081* by the method of Sauberlich and Baumann *(18)* with leucovorin (Lederle) as the standard of reference. Since leucovorin has half the activity of CF the results were halved to represent them as natural CF. The results are given in Table 1.

**Estimation of RNA.** Nucleic acids were extracted in the ungerminated and germinated seeds by the method of Schneider *(19)* and RNA was estimated by the method of Reeves and Munro *(16)*. Results are given in Table 2.

**TABLE 2**

**Ribonucleic acid contents of pulses (g. per 100 g.)**
**Figures are expressed in terms of the wet weight of the original seed taken for germination**

| Botanical name | Ungerminated | After germination | |
|---|---|---|---|
| | | 2 days | 4 days |
| *Cajanus indicus* ... | 5.33 ± 0.38 | 3.11 ± 0.13 | 2.89 ± 0.21 |
| *Cicer arietinum* .. | 6.99 ± 0.23 | 3.85 ± 0.17 | 4.25 ± 0.23 |
| *Lathyrus sativus* .. | 4.11 ± 0.38 | 3.26 ± 0.12 | — |
| *Phaseolus radiatus* | 5.14 ± 0.14 | — | 4.33 ± 0.16 |
| *Phaseolus mungo* . | 6.54 ± 0.14 | — | 6.44 ± 0.42 |

## RESULTS AND DISCUSSION

Of the different pulses studied and concentration of CF was comparatively high in *Cicer arietinum* and *Phaseolus radiatus*. CF values of all the pulses decreased during the process of germination. FA contents of pulses also decreased during germination *(2)*, and this decrease could not, therefore, be ascribed to its conversion to CF although ascorbic acid contents of germinated pulses were fairly high *(11)*. RNA contents of *Cicer arietinum* and *Phaseolus radiatus* were high when compared to other pulses. With the exception of *Phaseolus mungo* RNA contents diminished in all the pulses when they were germinated. FA and CF are cleaved to a diazotizable amine after incubation with liver slices and hogogenates *(7, 8)*. It might be possible that FA and CF are cleaved during the germinating process and thereby these contents are diminished in the germinating seeds.

## SUMMARY

Citrovorum factor and ribonucleic acid contents of some pulses *(Leguminous seeds)* were determined before and after they were germinated for 2 and 4 days. Pulses are good sources of these constituents. During the process of germination these values diminished. The diminished folic acid and citrovorum factor contents of germinated pulses might be due to cleavage of these substances during the germinating process.

**Acknowledgment**

Leucovorin and culture of *Leuconstoc citrovorum* were kindly supplied by Lederle Laboratories (India) Ltd.

## LITERATURE CITED

1. Banerjee, S., Rohatgi, K. S., Banerjee, M., Chattopadhyay, D. P., and Chattopadhyay, H. P. Pyridoxine, inositol and vitamin K contents of germinated pulses. *Food Research*, **20**, 545 (1955).

2. BANERJEE, S., ROHATGI, K. S., AND LAHIRI, S. Pantothenic acid, folic acid, biotin and niacin contents of germinated pulses. *Food Research*, **19**, 134 (1954).
3. CHATTOPADHYAY, H. P., AND BANERJEE, S. Effect of germination on the carotene content of pulses and cereals. *Science*, **113**, 600 (1951).
4. CHATTOPADHYAY, H. P., AND BANERJEE, S. Studies on the choline content of some common Indian pulses and cereals both before and during the course of germination. *Food Research*, **16**, 230 (1951).
5. CHATTOPADHYAY, H. P., AND BANERJEE, S. Effect of germination on the total tocopherol content of pulses and cereals. *Food Research*, **17**, 402 (1952).
6. CHATTOPADHYAY, H. P., NANDI, N., AND BANERJEE, S. Effect of germination on the thiamine content of pulses grown in Bengal. *Indian Pharmacist*, **5**, 121 (1950).
7. DINNING, J. S., SIME, J. T., WARK, P. S., ALLEN, B., AND DAY, P. L. Metabolic conversion of folic acid and citrovorum factor to a diazotizable amine. *Arch. Biochem. Biophys.*, **66**, 114 (1957).
8. FUTTERMAN, S., AND SILVERMAN, M. The inactivation of folic acid by liver. *J. Biol. Chem.*, **224**, 31 (1957).
9. LOWE, C. U., AND BARNUM, C. P. The synthesis of pentose nucleic acids in the liver of PGA deficient megaloblastic monkeys. *Arch. Biochem. Biophys.*, **38**, 335 (1952).
10. METALS, P., AND MANDEL, P. Action of aminopterin on the nucleic acids of the bone marrow of the rat; a quantitative study. *C. R. Soc. Biol.*, **149**, 1022 (1955).
11. NANDI, N., AND BANERJEE, S., Effect of germination on the vitamin C content of pulses grown in Bengal. *Indian Pharmacist*, **5**, 63 (1950).
12. NANDI, N., AND BANERJEE, S. Effect of germination on the riboflavin content of pulses grown in Bengal. *Indian Pharmacist*, **5**, 202 (1950).
13. NICHOL, C. A., AND WELCH, A. D. Synthesis of CF from FA by liver slices. Augmentation by ascorbic acid. *Proc. Soc. Exptl. Biol. Med.*, **74**, 52 (1950).
14. POHLAND, A., FLYNN, E. H., JONES, B. G., AND SHIVE, W. A. A proposed structure for folinic acid-SF, a growth factor. *J. Am. Chem. Soc.*, **73**, 3247 (1951).
15. ROHATGI, K. S., BANERJEE, M., AND BANERJEE, S. Effect of germination on vitamin $B_{12}$ values of pulses. *J. Nutrition*, **56**, 403 (1955).
16. REEVES, R. E., AND MUNRO, J. Determination of pentoses., *J. Industrial and Eng. Chem., (Anal. Ed.)*, **12**, 551 (1940).
17. SAUBERLICH, H. E., The effect of folic acid upon the urinary excretion of the growth factor required by Leuconstoc citrovorum. *J. Biol. Chem.*, **181**, 467 (1949).
18. SAUBERLICH, H. E., AND BAUMAN, C. A. A factor required for the growth of Leuconstoc citrovorum., *J. Biol. Chem.*, **176**, 165 (1948).
19. SCHNEIDER, W. C. Phosphorous compounds in animal tissues. *J. Biol. Chem.*, **161**, 293 (1945).

# EFFECT OF LOW TEMPERATURES ON THE PHYSIOLOGY OF GRAPEFRUIT *(CITRUS PARADISI MACFAD.)*

It is well known that temperatures below about 10° C., but above 0° C., may cause physiological disorders in tropical and subtropical fruits and storage organs of plants. It has been inferred that these physiological disorders are caused by interference with functions like respiration or with oxidizing reactions associated with respiration. Disturbance in respiration has been reported for papayas by Jones *(5)* and in cucumbers by Eaks and Morris *(3)*. Miller and Heilman *(7)* found physiological breakdown in pineapples to be associated with a loss of ascorbic acid and suggested that this resulted from disturbances in cellular oxidation.

Pitting of citrus fruits, a typical low temperature injury, has been the subject of many investigations *(6)*. Several of these have been limited to a study of the effect of environmental factors, such as relative humidity of storage rooms, or the use of waxes and impregnated wrappers on the fruits *(2, 9)*. Few have dealt with studies of changes in constituents. One of the first to suggest that certain changes in the constituents are responsible for pitting was Nelson *(8)* but he too confined his efforts to observations on environmental conditions. Nelson produced typical symptoms of pitting on grapefruit by storing them in an atmosphere of nitrogen. He concluded that the disorder is a manifestation of suboxidation. Harvey and Rygg *(4)* were the first to study changes in the composition of the peel of grapefruit during storage. They reported that hydrogen ion concentration of the peel increased during storage at 32°, 42°, and 52° F. Changes in naringin content were not consistent. The amount of pitting was greater when fruits were stored at 42° than at 32° or 52° F.

The purpose of the present investigation was to study the physiology of chilling injury in grapefruit, the approach being from a fundamental standpoint. Although several types of breakdown result from the chilling of grapefruit, "pitting" is perhaps the disorder most commonly encountered in commercial storage. Pitting is characterized by small indentations in the external surface of the rind. These sunken areas vary in size and shape. They may reach proportions of 10 mm. in diameter and 1 mm. in depth. The pits are seldom normal in color but range from yellow to dark brown.

## MATERIALS AND METHODS

Three different experiments were conducted during the commercial grapefruit season of 1957-58. A crate of Florida grapefruit, size 80, was selected for each experiment, two of them being Marsh seedless and the third a pink-fleshed (Thompson) seedless. Sixty uniform fruits were selected for each experiment. These were divided into 4 lots of 15 fruits each. One lot was analyzed immediately, the second was stored for 4 weeks at 10° C., the third for 4 weeks at 5° C. and the fourth was stored at 5° C. for 6 weeks. The 15 fruits in each lot were divided into 5 samples for analysis in order to compensate for individual variation.

Analyses were made of both segments and peel. Total acids were determined by extracting 100 g. of segments or peel with boiled distilled water and titrating with 0.10 and 0.01 normal sodium hydroxide respectively. Total acid was expressed as grams of citric acid per 100 g. of fresh peel or segments. The method used in determining reduced ascorbic acid content of the fruits was identical with that recommended by the Association of Vitamin Chemists *(1)*. This consists essentially of extracting the tissues with 6%

metaphosphoric acid and titrating with 2,6-dichlorophenolindophenol. The results were expressed as milligrams of ascorbic acid per 100 g. of fresh material.

Measurements of pH were made on duplicate 50 ml. aliquots of filtrate from the peels and segments with a glass electrode, using a Beckman pH meter.

## RESULTS

No consistent changes were noted in the total and ascorbic acid contents of the segments in any of these experiments. There was, on the other hand, a general tendency for total acid in the peel to increase during storage. (See Table 1). This occurred under all conditions in all experiments except in one instance; i.e., in the lot stored at 5° C. for 4 weeks in the second experiment. There was not only an increase in acidity in the remaining lots, but in most cases the difference was significant to the 1% level. Ascorbic acid likewise showed a tendency to increase during storage although the results were not so consistent as with total acids. An increase occurred under all conditions of storage in the first and third experiment only. Statistical significance was shown only in the lots of the first experiment.

**TABLE 1**

**Changes in total and ascorbic acids in the rinds of grapefruit during storage**

| Experiment No. | Original sample | 4 weeks at 10° C. | 4 weeks at 5° C. | 6 weeks at 5° C. |
|---|---|---|---|---|
| | **Total Acids (g. per 100 gms.)** | | | |
| 1 | 0.053 | 0.111 | 0.071 | 0.102 |
| 2 | 0.047 | 0.113 | 0.041 | 0.063 |
| 3 | 0.016 | 0.031 | 0.027 | 0.019 |
| Average | 0.035 | 0.085 | 0.046 | 0.061 |
| | **Ascorbic Acid (mg. per 100 gms.)** | | | |
| 1 | 83.6 | 114.5 | 95.4 | 132.1 |
| 2 | 156.8 | 149.7 | 137.1 | 153.4 |
| 3 | 190.8 | 195.2 | 200.0 | 204.0 |
| Average | 143.7 | 153.1 | 144.2 | 163.2 |

The pH of the extracts of peel ranged from 5.00 to 6.30 and those of the segments ranged from 3.50 to 3.75. There was no apparent change in the pH of the peel or segments in any of the stored lots.

Fruits which were stored at 10° C. for 4 weeks had developed a deeper yellow color and appeared a little shriveled. Those stored at 5° C. for either 4 or 6 weeks came out of storage with essentially the same color and firmness as before storage. All stored lots of fruit lost weight during storage, the loss in the 10° C. lots being greater than that of the 5° C. lots. (See table 2). None of these losses in weight was large enough to account for the observed increases in ascorbic or total acids.

**TABLE 2**

**Average loss of weight per fruit during storage**

| Experiment No. | Grams per day | | |
|---|---|---|---|
| | 4 weeks at 10° C. | 4 weeks at 5° C. | 6 weeks at 5° C. |
| 1 | 0.27 | 0.15 | 0.14 |
| 2 | 0.21 | 0.13 | 0.12 |
| 3 | 0.23 | 0.13 | 0.13 |
| Average | 0.24 | 0.14 | 0.13 |

The only instance in which definite symptoms of pitting appeared was in one lot of Marsh seedless stored at 5° C. for six weeks during the first experiment.

## DISCUSSION

It is not surprising that significant changes were found in the peel rather than in the segments because symptoms of physiological disorders of this type usually appear first in the peel of stored citrus fruits. Furthermore the pH of the peel (5.00 to 6.00) is closer to the optimum for oxidases than is that of segments (3.50 to 3.70).

Results indicate that storage at low temperatures may cause a suboxidation in the cells as was suggested by Nelson. This is borne out by the accumulation of ascorbic and total acids. Perhaps a temperature range of 0° to 10° C. interferes with respiration to such an extent that the process proceeds only fast enough to cause the accumulation of substances which are toxic to the organism. Whether the interruption occurs within, or just before the Krebs cycle, is not known. Accumulation of acids in the peel lends support to the latter explanation.

It should be profitable to extend these studies to include respiration rates of peel compared to segments and to quantitative determination of the activity of various oxidizing enzymes.

## SUMMARY

Florida grapefruit were stored for four weeks at 10° C. and for four weeks and six weeks at 5° C. Two lots of Marsh and one of Thompson seedless fruits were included in these studies. At the end of the storage period observations were made on loss of weight and on other physical changes. Peel and pulp were analyzed for pH, total and ascorbic acids.

No significant changes were observed in the constituents of the segments at the end of the storage periods. In general there was an increase in total acid of the peel in the stored grapefruit. Ascorbic acid in the peel increased in one lot of Marsh seedless fruits, decreased in the other, and increased in the peel of the Thompson seedless fruits during storage.

A possible explanation for the fundamental cause of pitting is offered.

### Acknowledgments

The authors are indebted to Ann Greenlee and Richard Hager for assistance with the experimental work in this project.

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## LITERATURE CITED

1. Association of Vitamin Chemists, Inc. *Methods of Vitamin Assay.* 1947. Interscience Publishers, Inc., New York, N. Y.
2. Brooks, Charles and McColloch, Lacy P. Some storage diseases of grapefruit. *J. Agr. Research,* **52,** 319-351 (1936).
3. Eaks, Irving L. and Morris, Leonard L. Respiration of cucumber fruits associated with physiological injury at chilling temperatures. *Plant Physiol.,* **31,** 308-313 (1956).
4. Harvey, E. M. and Rygg, G. L. Field and storage studies on changes in the composition of the rind of the Marsh grapefruit in California. *J. Agr. Research,* **52,** 747-787 (1936).
5. Jones, Winston W. Respiration and chemical changes of the papaya fruit in relation to temperature. *Plant Physiol.,* **17,** 481-486 (1942).

6. MILLER, ERSTON V. The physiology of citrus fruits in storage. Bot. Rev. **12**, 393-424. 1946. II *Bot. Rev.* **24**, 43-59. (1958).
7. MILLER, ERSTON V. AND HEILMAN, ALAN S. Ascorbic acid and physiological breakdown in the fruits of the pineapple (*Ananas cosmosus* L. Merr.). *Science*, **116**, 505-506 (1952).
8. NELSON, R. Some storage and transportation diseases of citrus fruits apparently due to suboxidation. *J. Agr. Research*, **46**, 695-713 (1933).
9. STAHL, ARTHUR L. AND CAIN, JOHN C. Cold storage studies of Florida citrus fruits. III. The relation of storage atmosphere to the keeping quality of citrus fruit in cold storage. Fla. Agr. Exp. Sta. *Bull.*, **316** (1937).

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